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Effect of pH and NaCl on Swelling and Drip in Fish Muscle

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ABSTRACT

Sodium chloride prevents drip in lightly brined fish muscle by causing the proteins to swell and to bind liquid firmly, and not by a surface sealing action. In whole and in comminuted muscle at its natural pH, free drip is almost completely inhibited, and expressible drip greatly reduced, by incorporation of about 1 per cent NaCl. On either side of an approximate "isoelectric zone" (about pH 4.5 to 6.0) addition of HCl or NaOH causes fish muscle to swell markedly and to withhold liquid against hydraulic pressure, peptization of the proteins occurring above and below about pH 10 and 2 respectively. Between about pH 4.5 and 7.0, from 1 to 3 per cent NaCl enhances swelling and liquid binding power of fish muscle, while below pH 4.5 it has an entirely opposite effect. Addition of NaCl effects an increase in pH of fish muscle below, and a decrease above about pH 4.5. In 1 per cent NaCl fish muscle does not swell appreciably; in 3 to 7 per cent swelling is at a maximum, while higher concentrations cause an apparent decrease in swelling which may be due in part to the peptizing action of the salt. Theoretical and applied aspects of the process of brining fillets are discussed.

It is well known that if, prior to freezing, fish muscle is briefly immersed in sodium chloride brine, the free exudation of liquid, or drip, which normally occurs when such tissue is defrosted (thawed) can be largely or entirely prevented (Taylor 1929, 1932, 1933; Reay 1931, 1933a; Beatty 1931; Tressler and Murray 1932). Such treatment has been widely adopted by commercial firms on this continent, large quantities of fillets and steaks being lightly brined prior to freezing. During the past year a fairly thorough study of the process has been made at this Station in connection with investigations on fillet preservation, and it has been found that free drip from unfrozen fillets as well as from fillets cut from defrosted fish, can be prevented by the incorporation of slightly over 1 per cent of sodium chloride in the flesh through brief immersion in a suitably concentrated brine (Tarr 1940, 1941a; Tarr and Sunderland 1940a and b).

METHODS

Halibut muscle was used in these experiments because it was readily available and is fairly prone to drip formation (Tarr 1940, 1941a; Tarr and Sunderland 1940a). Partly for convenience, and partly for the sake of comparison, experiments have been carried out in some instances with unfrozen, and in others with defrosted, muscle. The frozen fish used were obtained from local cold storage companies. They had been frozen in air in sharp freezing rooms, glazed and

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stored for some months at fairly low temperatures (probably at about −20°C.). They were defrosted in air at about 18 to 20°, the temperature of the flesh not being permitted to rise above 10°. No attempt was made to select fish of uniform size, the weight varying between 5 and 12 kg. Comminuted muscle was prepared by passing the flesh through the coarse plates of an ordinary domestic mincing machine, the muscle of frozen fish being minced before it was completely defrosted in order to avoid loss of fluid.

"Equilibrium" extraction of finely minced muscle with three volumes of cold distilled water, and titration by Mohr's method (White 1939), was the procedure adopted for the determination of NaCl. A Beckman pH meter with glass and calomel electrodes was employed to determine pH, all measurements being made at 20°C.

Measurement of Free and Expressible Drip

In this paper free drip is understood to mean the liquid which muscle tissue exudes on standing without the application of appreciable mechanical pressure. Reay (1934) considers that the protein in free drip is mainly myosin sol. By expressible drip (press juice) is understood that fraction which is obtained by subjecting muscle tissue to hydraulic pressure. There is no standard technique for measuring drip from either meat or fish tissue, each worker having invented or adopted a method which appeared to best suit his requirements. It is therefore inevitable that there is little consistency in reports stating the quantity of drip obtained from a given tissue. Indeed recent experiments by Young (1941) have revealed the fact that important differences may occur in the quantity of free drip exuded by closely adjacent samples from the same fish. The methods employed for measuring drip in these experiments are recorded herewith.

free drip

In brining experiments weighed pieces of muscle are immersed in brine for a given time, and then drained for 5 minutes at 18 to 20°C. on a sloping wire screen. They are then placed in weighed, sterile 600-ml. beakers covered with an inverted half of a petri dish and, after determining any gain in weight due to the brining procedure, stored at the desired temperature. Unbrined samples are treated similarly. Under such conditions evaporation at about 1.5° is very slight, and even at 20° for 3 days usually results in a decrease of only about 1 per cent in the weight of the sample. The loss of weight as drip is determined at frequent intervals by weighing the units after draining off all the liquid which has accumulated. This method has been found to give quite satisfactory results, and is especially useful in cases where bacteriological analyses are required, since no gross external contamination can occur.

expressible drip

The samples to be examined (200 to 300 g. of whole or comminuted muscle in different experiments) are placed in dry, weighed 38×38 cm. press cloths made from no. 8 canvas duck and subjected for ten minutes to a pressure of approximately 5000 lb. per square inch (3618 kg. per sq. cm.) in a Carver hydraulic press.

Since a large proportion of the expressed liquid remains on the press cloth the following arbitrary method has been adopted in determining total expressible drip. The freely expressed liquid is collected and its weight determined, the muscle is carefully scraped from the press cloth and weighed, and the amount of liquid remaining on the cloth itself calculated by difference. Records have shown that usually at least 99 per cent of the weight of the muscle pressed can be accounted for in this way. The results obtained are, however, useful only for purposes of comparison. The figures for total expressible drip naturally tend to be high; for frequently, and especially in case of salt or alkali treated muscle, a good deal of tissue is pressed into the pores of the cloth and cannot be readily removed and is thus accounted for as expressed fluid.

EXPERIMENTS WITH WHOLE MUSCLE

Penetration of NaCl and Prevention of Drip

Apparently the view is sometimes held that brining prevents drip in fillets as a result of a coagulation or "case hardening" of the surface proteins, and that the layer so formed prevents the exudation of liquid. This impression has probably arisen from the fact that the brining process causes the surface of fillets to acquire a viscous glossy layer of peptized protein which normally dries to a shiny "pellicle" when they are smoked (Reay 1934). Though this layer of peptized protein may retard the penetration of sodium chloride it would seem that it plays little or no part in preventing drip. This is borne out by the following experiments, and also by results of work already published (Tarr and Sunderland 1940a) in which it was shown that free drip in fresh halibut fillets is not entirely prevented until the brining procedure employed is such that about 1.3 per cent NaCl is incorporated in the flesh.

EXPERIMENT 1

Seven pieces of muscle approximately 7.5×5×2.5 cm. in size were cut from a defrosted halibut. One was retained untreated as control, the other six being brined for 2 minutes in 2 litres of 20 per cent (by volume) NaCl solution at 0 to 1°C. The NaCl content of one of the brined samples, selected at random, was 1.1 per cent. Of the five remaining brined pieces one was not treated further, while the other four were cut parallel to the largest surface into two approximately equal pieces at intervals of 5, 20, 30 and 60 minutes after removing them from the brine. After cutting, the surfaces which had been exposed to the brine were placed together in order that the freshly cut surfaces should have full opportunity to exude liquid. The quantity of free drip formed by each of these samples in 24 hours at 20° was determined as already described. A storage temperature of 20° was chosen because drip formation proceeds more rapidly and to a greater extent at high than at low temperatures (Tarr 1941a). The results of this experiment, given in table I, showed quite definitely that the effect of brining in preventing free drip could not be accounted for by a "surface sealing", for the amount of liquid which the cut brined fillets exuded was only very slightly greater than that from the uncut brined fillet. The slight, and probably insignificant, differ-

ences in the amount of drip formed in fillets cut at various intervals subsequent to brining indicated that the salt penetrated sufficiently rapidly to bring about the changes in the proteins which prevent drip (*vide infra*).

TABLE I. The influence of cutting subsequent to brining on the quantity of free drip obtained from defrosted halibut muscle.

Treatment	*Wt. of muscle used (brined or unbrined)	Loss of wt. as drip (g.)	Loss of wt. as drip (%)
Unbrined	365.1	73.6	20.2
Brined, uncut	342.8	2.5	0.7
Cut 5 min. after brining	311.0	10.3	3.3
Cut 20 min. after brining	354.0	6.7	1.9
Cut 30 min. after brining	339.6	11.2	3.3
Cut 60 min. after brining	343.8	6.2	1.8

*Muscle brined under similar conditions usually increases about 3 to 4 per cent in weight (Tarr 1940, 1941a).

EXPERIMENT 2

Five pieces of muscle of about the same size and weighing approximately 200 g. (see table II) were cut from a defrosted halibut. One piece was retained as control, the remaining four being immersed for 2 minutes in 20 per cent (by volume) NaCl brine at 0 to 1°C. and drained as usual. The expressible drip was determined immediately subsequent to cutting in the case of the control (unbrined) sample, whereas the brined samples were stored at 1.5° and pressed at various intervals. A similar experiment was performed with defrosted muscle from another fish, the samples in this case being immersed for 1 minute in 15 per cent (by volume) sodium chloride brine which caused them to absorb approximately 1.0 per cent of the salt (table III). It is evident from the results of these experiments that the amount of expressible drip decreased as the interval between brining and pressing increased. This indicates that the action of the brine was not purely superficial, but that the salt slowly penetrated the flesh, giving rise to some alteration in the proteins which caused them to bind liquid firmly.

TABLE II. Influence of length of time after brining on quantity of expressible drip from defrosted halibut muscle. (NaCl concentration in muscle about 2.8 per cent).

Treatment	Expressible drip collected (g.)	Expressible drip on cloth (g.)	Total expressible drip (g.)	Total expressible drip (% of 200-g. sample)
Unbrined	26	54	80	40
Pressed 10 min. after brining	11	51	62	31
Pressed 60 min. after brining	7	39	46	23
Pressed 6 hr. after brining	0	30	30	15

TABLE III. Influence of length of time after brining on quantity of expressible drip from defrosted halibut muscle. (NaCl concentration in muscle about 1 per cent).

Treatment	Expressible drip collected (g.)	Expressible drip on cloth (g.)	Total expressible drip (g.)	Total expressible drip (% of 200-g. sample
Unbrined.....	35	60	95	48
Pressed 5 min. after brining.	21	52	73	36
Pressed 1 hr. after brining..	14	46	60	30
Pressed 6 hr. after brining..	10	51	61	30
Pressed 24 hr. after brining.	9	45	54	27

EFFECT OF NACL CONCENTRATION ON FREE AND EXPRESSIBLE DRIP

Twelve pieces of muscle about 10×7.5×4.5 cm. in size (271.7 to 281.8 g. in weight) were cut from a defrosted halibut. Two were retained untreated as controls, the remaining ten being brined in pairs for various lengths of time in 15 per cent (by volume) NaCl brine at 0 to 2°C., the gain in weight due to brining being determined. One piece from each treatment was used to measure expressible drip, and the other to measure free drip. The samples used to determine free drip were subsequently employed for estimation of the amount of NaCl in the muscle. The results (table IV) show that there was a progressive decrease in both free and expressible drip as the salt concentration in the muscle increased. Free drip was practically eliminated by 1.17 per cent NaCl, and this result agrees closely with previous findings where, in the case of muscle from unfrozen halibut, 1.29 to 1.33 per cent NaCl entirely prevented free drip (Tarr and Sunderland 1940a). Expressible drip was considerable in samples containing sufficient NaCl to practically eliminate free drip. This is not surprising since it was previously shown that even 2.8 per cent NaCl did not entirely prevent expressible drip (table II).

TABLE IV. Influence of NaCl content of defrosted muscle on amount of free and expressible drip.

Brining time (seconds)	NaCl in muscle (%)	Expressible drip collected (g.)	Expressible drip on cloth (g.)	Total expressible drip		†Free drip (%)
				(g.)	*(%)	
0	0.09	52	59	111	44	−17.6
10	0.70	41	61	102	41	−10.7
30	0.75	34	54	88	35	−9.0
60	0.82	29	47	76	30	−6.6
120	1.14	23	50	73	29	−3.0
300	1.17	27	45	72	29	+0.1

*Calculated on the weight of the brined or unbrined muscle.

†Brined samples gained from 0.2 to 3.0 per cent in weight depending on period of immersion. However, the figures for loss (−) or gain (+) in weight due to drip were calculated from the difference in weight of the unbrined muscle and that obtained after the drip liquid was decanted.

SWELLING IN DILUTE HCl OR NaOH WITH AND WITHOUT NaCl

Six pieces of muscle about 5×5×2.5 cm. in size (97.5 to 101.5 g.) were cut from an unfrozen halibut which has been stored 5 days in ice. One piece was placed in each of the following solutions contained in 600-ml. beakers covered with an inverted petri dish.

No. 1. 20 ml. 1.0 N. HCl+water to 400 ml.
" 2. " " " " " +8 g. NaCl*+water to 400 ml.
" 3. 400 ml. of distilled water
" 4. 8 g. NaCl*+water to 400 ml.
" 5. 6 ml. 1.0 N. NaOH+water to 400 ml.
" 6. " " " " " +8 g. NaCl*+water to 400 ml.

*NaCl sufficient to make a final concentration of 2 per cent by volume.

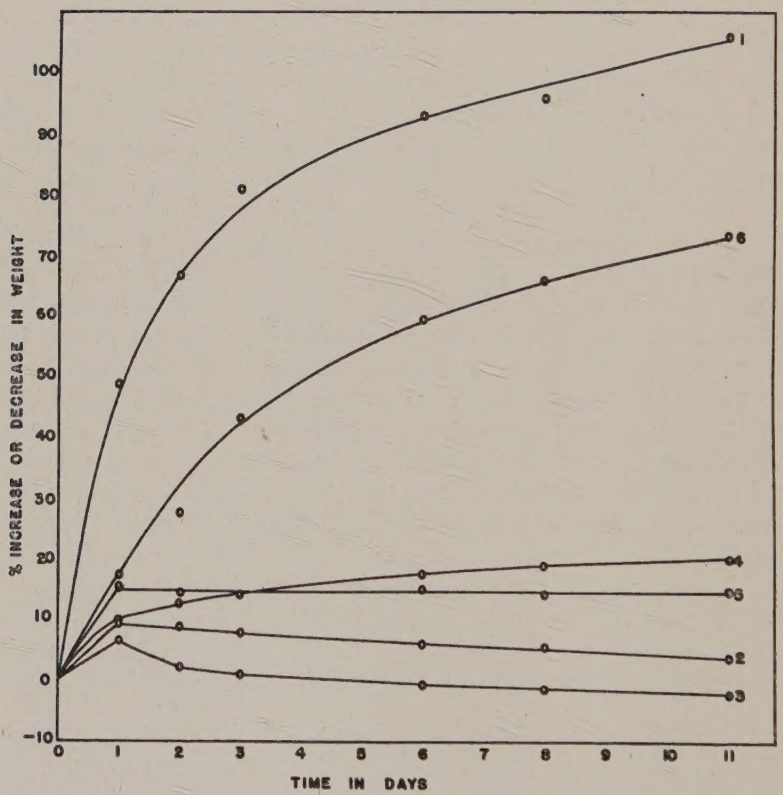

FIGURE 1. Swelling of whole unfrozen halibut muscle at different pH with and without 2% NaCl. 1. Muscle treated with HCl alone; final pH of liquor, 3.60. 2. Muscle treated with HCl+2% NaCl; final pH of liquor, 3.50. 3. Muscle treated with water alone; final pH of liquor, 6.22. 4. Muscle treated with water+2% NaCl; final pH of liquor, 6.26. 5. Muscle treated with NaOH alone; final pH of liquor, 7.40. 6. Muscle treated with NaOH+2% NaCl; final pH of liquor, 6.88.

The containers were stored at approximately 1.5°C., the solutions being drained off at intervals and the samples weighed (to the nearest 0.5 g.), after which the liquid was replaced and the containers again placed in storage. After 11 days, when a pronounced stale odour developed in certain of the samples, the experi-

ment was concluded, the pH of the liquid being determined in each instance. It will be seen (figure 1) that swelling was extremely marked in HCl solution alone, but that under these conditions the presence of NaCl very strongly inhibited swelling. In distilled water alone there was at first a slight initial swelling which was not maintained, while in NaCl solution alone there was moderate swelling which slowly increased during storage. Moderate swelling also took place in dilute NaOH solution, and this was increased enormously by the addition of NaCl.

EXPERIMENTS WITH COMMINUTED MUSCLE

Influence of pH on Swelling

Fifty grams of minced muscle from a defrosted halibut were placed in each of sixteen 250-ml. centrifuge bottles which were immersed in ice water. To each sample 200 ml. of water alone or water with the addition of various amounts of acid or alkali (table V) were added, the temperature of the liquid being about 1.5°C. in each case. After mixing the solutions and muscle thoroughly, the containers were stored at approximately 1.5° for 1 day. They were then centrifuged

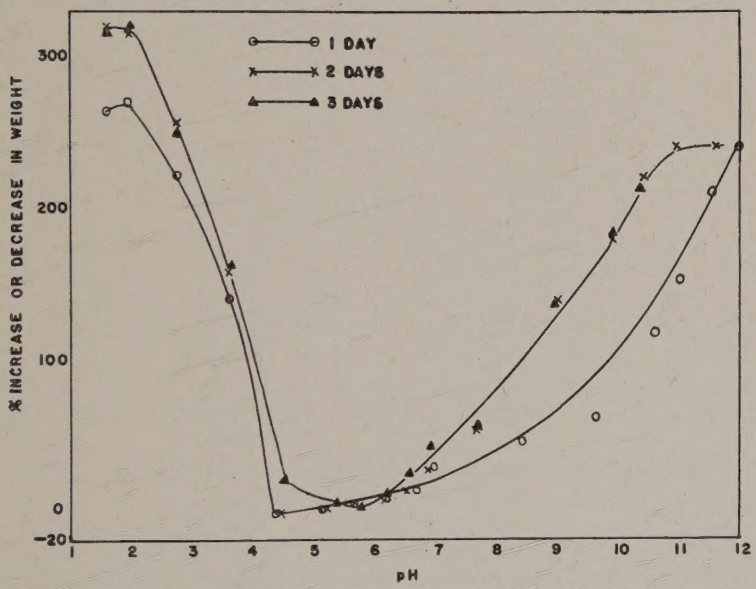

Figure 2. Influence of pH and time on swelling of comminuted, defrosted halibut muscle.

at about 18 to 20° for 10 minutes at approximately 2000 r.p.m. on a centrifuge having an average effective radius of 12 cm. The supernatant liquid was carefully decanted from the sample and measured, and from this the increase in weight of the muscle was calculated. The supernatant liquid was then mixed with the muscle, the pH determined, and the samples returned to storage. They were examined similarly at intervals of 2 and 3 days after the commencement of the experiment. It will be seen (table V and figure 2) that a stage approximately that of equilibrium both as regards swelling and change in pH was reached by the second day, no significant change occurring between the second and third day of

storage. Therefore, in subsequent experiments of this sort a 2-day storage period was used. It will be observed that below about pH 4.5 and above about pH 6 the muscle swelled very definitely. Between these two pH values there is an approximate "isoelectric zone" (Loeb 1924), in which no appreciable swelling occurs. At values of pH above about 10 and below about 2 the muscle proteins were markedly peptized; the solutions became viscous and the muscle tissue itself very gelatinous, and consequently the results obtained at these extremes are only very approximate.

TABLE V. Influence of pH and time on swelling of comminuted defrosted halibut muscle.

Ml. of acid or base added		pH (20°C.) of sample after			Increase or decrease (%) in weight after		
1.0 N HCl	1.0 N NaOH	1 day	2 days	3 days	1 day	2 days	3 days
25		1.60	1.60	1.60	264	320	318
20		1.95	1.95	2.00	270	316	320
15		2.75	2.75	2.75	222	256	250
10		3.60	3.60	3.65	140	158	161
5		4.35	4.45	4.50	−2	−2	10
2		5.10	5.20	5.35	0	0	4
1		5.65	5.65	5.75	4	4	2
0		6.20	6.15	6.20	6	6	10
	1	6.65	6.50	6.55	12	12	24
	2	6.95	6.85	6.90	28	26	42
	4	8.40	7.65	7.65	44	52	54
	6	9.60	9.00	8.95	60	138	136
	8	10.60	9.90	9.85	116	178	182
	10	11.00	10.40	10.35	152	220	206
	*12	11.55	10.95	10.80	210	240	..
	*16	12.00	11.60	11.45	240	240	..

*At high pH values the proteins are rapidly peptized and the results are only very approximate.

INFLUENCE OF NaCl ON SWELLING AT DIFFERENT pH

FRESH MUSCLE

Fifty grams of comminuted muscle prepared from an unfrozen halibut stored for 5 days in ice were placed in each of twenty 250-ml. centrifuge bottles which were immersed in ice water. To each sample was added 200 ml. of water, or solution of acid or alkali, with or without sufficient NaCl to cause the mixture to contain 3 per cent of this salt (table VI). After mixing thoroughly the samples were stored for 2 days at about 1.5°C., the muscle and liquid having been mixed after storage for one day. The amount of swelling and pH were determined after 2 days as in the foregoing experiment. The results (table VI and figure 3) show that below about pH 4.5 the swelling due to acid alone was almost entirely suppressed by 3 per cent NaCl. Between pH 4.5 and 7, NaCl had an entirely opposite effect, causing an increase in swelling greater than that occasioned by alkali alone at any pH. From pH 7 to about 8.5 the addition of NaCl did not further

enhance swelling, and thereafter progressive increase in pH caused an apparent decrease in swelling. This is probably due to the peptizing action of NaCl at the high pH values. If a pH of about 4.5 is taken as the approximate isoelectric point of the proteins, then it may be stated that above this point NaCl promotes, and below it suppresses, swelling. It is also interesting that NaCl effected a decrease in pH on the alkaline side, and an increase in pH on the acid side of this hypothetical isoelectric point.

TABLE VI. Influence of pH and NaCl (3 per cent) on swelling of comminuted fresh halibut muscle.

Vol. of 1.0 N acid or base added (ml.)	Sample	pH of sample at 20°C.	Increase or decrease in weight (%)
20	Control	1.65	136
	NaCl	1.90	16
15	Control	2.40	108
	NaCl	2.75	10
10	Control	3.35	82
	NaCl	3.60	0
5	Control	4.20	−6
	NaCl	4.50	8
0	Control	6.50	8
	NaCl	6.15	112
1	Control	6.85	20
	NaCl	6.50	124
2	Control	7.35	26
	NaCl	7.00	130
4	Control	9.20	64
	NaCl	9.10	128
6	Control	10.30	100
	NaCl	9.95	90
8	Control	10.95	*
	NaCl	10.45	*

*Viscous, peptized protein rendered measurement of swelling impossible.

DEFROSTED MUSCLE

An experiment similar to the foregoing was carried out with muscle from a defrosted halibut. The results, which are given in figure 4, show that defrosted muscle behaved similarly to fresh muscle except that the degree of swelling was

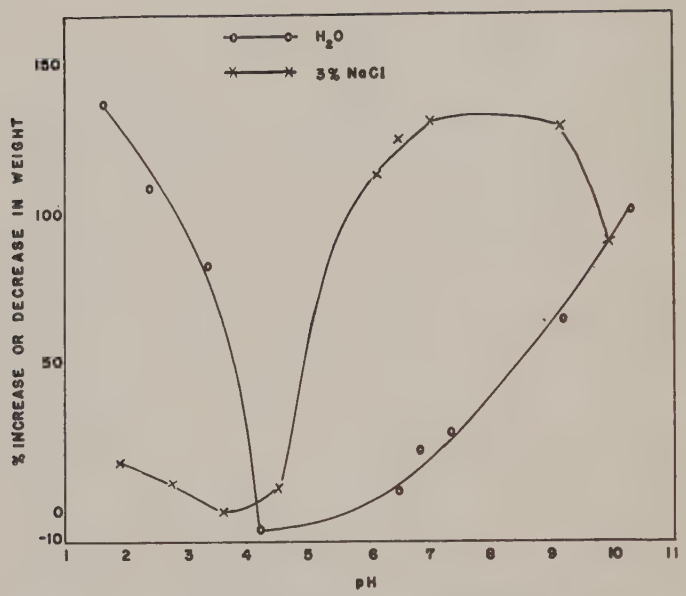

FIGURE 3. Influence of pH and NaCl concentration on swelling of comminuted, unfrozen halibut muscle.

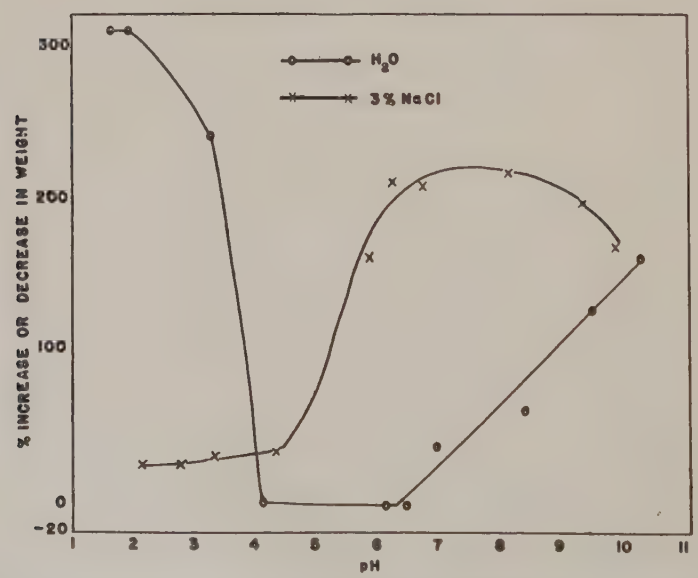

FIGURE 4. Influence of pH and NaCl concentration on swelling of comminuted, defrosted halibut muscle.

very much more pronounced. Whether this is due to an actual increase in the liquid-imbibing power of the proteins, caused, perhaps, by denaturation during freezing and storage, or merely to a chance sampling difference due to variations in individual fish, remains to be determined.

Influence of NaCl Concentration on Swelling

This determination was complicated by the fact that increasing concentrations of NaCl caused an increase in the change in pH which this salt normally occasions, and that the increase in swelling due to NaCl occurred over a relatively wide range of pH. In one experiment the pH was made practically uniform and slightly above 7 by varying the amount of alkali added to the muscle. and in another no attempt was made to stabilize the pH.

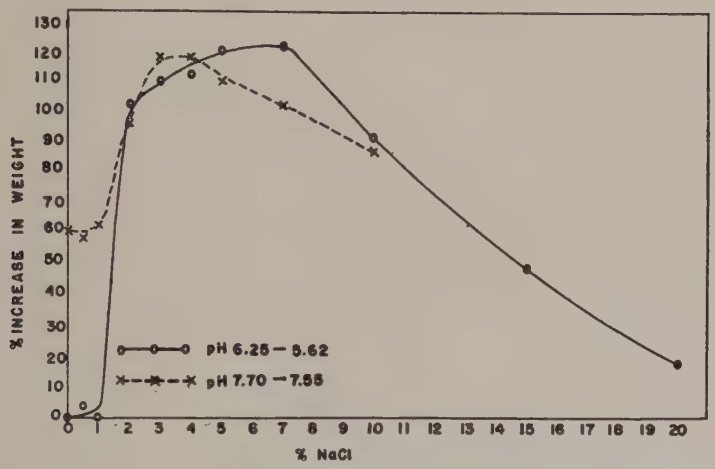

Figure 5. Influence of various NaCl concentrations on swelling of defrosted halibut muscle.

pH adjusted

Fifty-gram portions of comminuted, defrosted halibut muscle were placed in each of ten 250-ml. centrifuge bottles, 1 N NaOH and 30 per cent (by volume) NaCl being added in varying amounts in 100 ml. of solution (table VII) so that the pH was maintained at a practically constant level while the NaCl concentration varied. The temperature of both muscle and solutions was maintained at about 1.5°C. as in previous experiments, and after 2 days the amount of swelling and pH were determined as usual. The results (table VII and figure 5) showed that at this fairly constant pH (7.55 to 7.70) the increase in swelling due to the alkali alone was not appreciably affected by NaCl in concentrations of 1 per cent or lower. In higher concentrations of NaCl swelling increased very sharply to a maximum at 3 to 4 per cent of this salt, while above this level there was an apparent decrease in swelling, due, presumably, to the peptizing action of the salt.

TABLE VII. Influence of NaCl concentration on swelling of comminuted muscle, pH 7.55 to 7.70

NaCl added (%)	0.0	0.5	1.0	2.0	3.0	4.0	5.0	7.0	10.0
Vol. of 1.0N NaOH added (ml.)	4.0	4.05	4.10	4.15	4.20	4.25	4.30	4.60	4.80
pH at 20°C	7.65	7.65	7.60	7.65	7.70	7.65	7.55	7.55	7.70
Increase in weight (%)	62	60	64	98	120	120	112	104	94

pH NOT ADJUSTED

A similar experiment was performed with muscle from the same fish, but with no attempt to adjust the natural pH which fell markedly on the addition of NaCl. The results (table VIII and figure 5) differed only very slightly from those obtained in the foregoing experiment. Thus 1 per cent NaCl caused no swelling; concentrations of between 2 and 7 per cent occasioned very marked swelling; higher concentrations causing an apparent decrease.

TABLE VIII. Influence of NaCl concentration on swelling of comminuted muscle, pH 5.62 to 6.25

NaCl added (%)	0.0	0.5	1.0	2.0	3.0	4.0	5.0	7.0	10.0	15.0	20.0
pH at 20°C	6.25	6.12	6.05	5.98	5.96	5.94	5.90	5.84	5.75	5.65	5.62
Increase in weight (%)	0	4	104	104	112	114	122	124	94	50	18

EFFECT OF NACL CONCENTRATION ON EXPRESSIBLE DRIP

Muscle from an *unfrozen* halibut stored for 2 days in ice and also separately from a *defrosted* fish was minced and stored for 2 days at 1.5°C. during which time much free drip formed. The mince was thoroughly mixed with the drip liquid and 300-g. or 200-g. portions placed in each of four 600-ml. beakers. To each sample 20 ml. of water, or NaCl solution to give various concentrations (table IX) of this salt in the mince, were added and intimately mixed with the muscle. After standing for 10 minutes at about 20° each sample was subjected to hydraulic pressure in the usual manner. The results (table IX) show that for the defrosted as for the unfrozen muscle increasing concentrations of NaCl up to 1 per cent caused marked diminution in the quantity of expressible drip, but that above this value no further decrease occurred.

TABLE IX. Influence of NaCl concentration on amount of expressible drip from unfrozen and defrosted comminuted muscle.

NaCl in sample (%)	Expressible drip collected		Expressible drip on press cloth		Total expressible drip		Expressible drip	
	Unfrozen (g.)	Defrosted (g.)	Unfrozen (g.)	Defrosted (g.)	Unfrozen (g.)	Defrosted (g.)	Unfrozen (%)	Defrosted (%)
0.0	55	39	53	55	108	94	34.8	44.8
0.5	11	21	49	49	60	70	18.3	33.4
1.0	1	1	35	32	36	33	11.3	15.7
1.5	..	0	..	30	..	30	....	14.3
2.0	0	..	37	..	37	..	11.6	....

Influence of pH on Expressible Drip with and without 1% NaCl

Six 200-g. portions of comminuted muscle prepared from a halibut still in *rigor* were placed in 600-ml. beakers and intimately mixed with different solutions having the composition given in table X. After storing for 1 day at approximately 1.5°C. the pH of each sample was determined and then the amount of expressible drip. The results of this experiment (table X) show that the ability of muscle to hold liquid against pressure was greatly enhanced by neutralizing its natural acidity with dilute NaOH solution (to pH 7.15), and that the simultaneous addition of 1 per cent NaCl caused an even more marked retention of liquid. At the natural pH of muscle (6.2) liquid was but poorly retained, the addition of NaCl improving retention. Treatment with HCl (to pH 3.5) caused a decrease in the amount of expressible drip. In this case, however, the simultaneous addition of NaCl completely neutralized the effect of the acid treatment, the liquid-retaining power of the muscle being very much less than that of untreated muscle.

Table X. Influence of pH with and without 1% NaCl on amount of expressible drip from comminuted muscle.

Solution	pH at 20°C.	Expressible drip (g.)	Expressible drip on cloth (g.)	Total expressible drip (g.)	*Total expressible drip (%)
50 ml. water	6.20	75	55	130	52
50 ml. water+2.5 g. NaCl	6.15	5	42	47	18.8
50 ml. 0.2 N NaOH	7.15	45	58	103	41.2
50 ml. 0.2 N NaOH+2.5 g. NaCl	7.10	0	30	30	12.0
50 ml. 1.0 N HCl	3.45	60	53	113	44.6
50 ml. 1.0 N HCl+2.5 g. NaCl	3.50	103	54	157	62.8

*Calculated on 250 g. (i.e. wt. of fish+wt. of liquid).

DISCUSSION

It must be emphasized that the observations recorded in this paper have been made on whole muscle, which contains not only a variety of proteins (myogen, myosin, collagen, etc.) and possibly their breakdown products, but also other organic compounds (e.g. lactic acid, glycogen, trimethylamine oxide, sugar phosphates, etc.) and small amounts of inorganic salts, all of which may play some part in the effects observed. It would, however, seem fairly safe to assume that the alterations involving the swelling and liquid-binding power of the muscle are largely due to certain of the proteins present, especially in view of the fact that there is a pronounced isoelectric zone in which these effects are least pronounced.

It has been demonstrated quite definitely that the prevention of drip in fish muscle (unfrozen or defrosted) occasioned by a brief immersion in NaCl brine is not merely a superficial phenomenon resulting from a "surface sealing", but a deep-seated one, apparently depending largely on the effect of NaCl in enhancing the liquid-binding power of the muscle proteins. It has been found that there is

an approximate isoelectric zone (about pH 4.5 to 6.0), above or below which very marked swelling and retention of liquid occur. Similar, though more marked, swelling has been observed in meat connective tissue by Moran and Hale (1929), who stated that the collagen fibres are responsible. In the case of fish muscle above about pH 10 or 11, or below pH 2, the proteins are so markedly peptized that accurate measurement of swelling is not possible. NaCl in concentrations of from 1 to 3 per cent has a very marked effect on the swelling and liquid-binding power of the muscle at different pH. Thus there is a rather definite point (about pH 4.5) above which, and within certain limits, it enormously enhances these properties, but below which it has an exactly opposite effect.

These facts suffice to explain the slight gain in weight (3 to 4 per cent) which results when fillets are briefly immersed in fairly strong brines (Tarr 1940, 1941a). They also explain why treatment of fish flesh with organic acids in order to preserve it (Nadeau 1939) produces very undesirable physical changes (Tarr 1940). Such a procedure first occasions extensive superficial swelling with formation of a gelatinous layer. Then as the acid penetrates the flesh and equilibrium is established (about pH 5) the proteins are brought near the isoelectric point at which ability to retain liquids is at a minimum, and consequently extensive drip occurs. Any attempt to brine such fillets in the hope of preventing drip would naturally be valueless.

The results obtained in this work are of interest in that they to some extent verify and extend the original observations of Taylor (1929, 1933) regarding the conditions essential for retention of liquid by muscle. He recognized that neutralization of the natural acid formed in muscle during *rigor* effectively prevented drip, and was granted certain patents for processes in which the muscle was treated with alkaline brines in order to bring it to approximately pH 7. Later both Empy (1933) and Finn (1934) realized the possibilities of this type of treatment, but it is not clear whether it has ever received any commercial application. As the writer has pointed out, neutralization of the natural acidity of muscle favours bacterial growth and a fairly prolonged treatment is required even to alter the pH slightly (Tarr 1941b). Taylor (1929, 1933) advocated the addition of NaCl (10 per cent) to the alkaline solutions used for treating muscle in order that by creating an exosmosis it might overcome the swelling due to the alkali alone. It would appear unlikely that NaCl would have this effect since the present work has shown that NaCl tends to promote swelling at pH 7. That NaCl and pH are perhaps by no means the sole factors which will prevent drip in fish muscle is indicated by the work of Tressler and Murray (1933) who claim to have controlled it in both meats and fish by treatment with certain proteolytic enzymes. These investigators do not describe the mode of action of such enzymes. The mechanism by which drip is formed in muscle is by no means fully understood, though it seems that the thixotropic myosin gel is gradually transformed into a free-flowing sol. It would appear that NaCl and alkali treatment prevent drip by causing certain muscle proteins to swell and imbibe free liquid, though it is possible that they tend to make the myosin sol undergo gelation.

It has been shown that swelling of muscle is greatest in from 3 to 7 per cent NaCl solution, depending on the pH. In this connection it is of interest that

Reay (1936) showed that increase in weight in herring fillets was at a maximum in 4.2 to 9.0 per cent NaCl solution after 10 days.

No such curious action as that of NaCl in enhancing swelling above an apparent isoelectric point (pH about 4.5), and almost or totally inhibiting it below this point, appears to have been described. Loeb (1924, p. 2) stated that "The addition of little acid or alkali to originally isoelectric protein increases . . . the swelling of protein gels. The addition of neutral salts has only a depressing effect on these properties." It is obvious that this statement regarding the action of neutral salts does not hold in the case of whole fish muscle, nor always in the case of other proteins as the following references show. Jordan-Lloyd and Pleass (1927) found that in the case of purified gelatin 0.2 M NaCl actually enhanced swelling in a small isoelectric zone between pH 3.5 and 6.0. Thompson (1933) distinguished (in the case of gelatine) "osmotic swelling", which is presumably due to the Donnan membrane equilibrium theory of swelling as proposed by Procter and Wilson (Loeb 1924), from the relatively slight "lyotropic swelling" which occurs at the isoelectric zone. That very small amounts of certain salts may cause a pronounced effect on swelling is shown by the work of Lillie (1921). He found that the gelatinous membrane of starfish (*Asterias forbesi*) eggs taken from sea water swelled very markedly in isotonic NaCl solution, and was eventually peptized. The swelling was prevented by 0.0025 M $CaCl_2$.

Evidently swelling in the case of pure proteins cannot be adequately explained on the basis of a single theory, and the difficulty of interpreting the effects as observed in fish muscle is therefore apparent. It is possible that isolation of the myosin (Edsall 1930) and other protein fractions from fish muscle and a separate study of these will help to explain some of the effects observed. Reay (1931) has stated that it is chiefly the globulin (myosin) fraction of fish muscle which is denatured on freezing, so that if this fraction plays any significant part in the phenomena observed in the experiments recorded in this paper, then denaturation would not appear to lessen its activity appreciably.

These experiments have practical implications. It has been found that a brief brining of fish muscle causes it to increase significantly in weight, a gain of 2 to 4 per cent being common. This is not entirely desirable for obvious reasons. On the other hand brining prevents the enormous losses in soluble proteins which would otherwise occur. Reay (1931), who was one of the first to investigate the possibilities of this process, did not consider that brining was entirely necessary or desirable. He found that denaturation due to freezing proceeded slightly more rapidly in brined than in unbrined muscle, and concluded that, providing fish is frozen rapidly when strictly fresh and stored under optimum conditions, brining should not be necessary because drip is not significant in fish so treated. Unfortunately such conditions are seldom realized commercially, and in any case some drip will occur if fillets are held for any length of time after defrosting. For these reasons the brining process has been widely applied. Tressler and Murray (1932) advocated brining fillets, but recognized the need for caution. They showed that the use of impure salt resulted in the formation of a "salt fish flavour" (rancidity) during storage in the frozen state, especially when the flesh was exposed to ultraviolet light, and stated that only pure salt should be used. It is now well known

that NaCl treatment of fish flesh is liable to promote rancidity (Banks 1937). For this reason it is advocated that brined fillets, especially those cut from fish susceptible to rancidity, be stored for only a limited period, and preferably at a very low temperature (e.g. −30°C.). Brining distinctly improves the appearance of treated fillets owing to the fact that a shiny layer of peptized protein (myosin) forms on the surface (Reay 1933a and b), and it is sometimes held that the small amount of salt incorporated enhances the natural flavour of the fish. It has been shown that fillets which have been subjected to a brief immersion in fairly strong brine keep better than those not so treated (Tarr 1940).

SUMMARY

Experiments involving measurement of free and expressible drip have shown that the prevention of drip occasioned by lightly brining unfrozen and defrosted fish muscle at its natural pH lies almost, or entirely, in the ability of NaCl to cause the proteins to bind liquid firmly, and is not due to any purely superficial sealing effect of the layer of peptized protein (myosin) which forms on the surface.

Free drip and expressible drip both decreased as the NaCl concentration of whole muscle increased up to approximately 1 per cent, at which value free drip was only very slight. Expressible drip was considerably reduced but by no means entirely eliminated by this or higher concentrations of NaCl (e.g. 2 to 2.8 per cent).

Whole muscle swelled gradually in dilute HCl or NaOH solution, the swelling in acid being almost entirely inhibited by NaCl and that in alkaline solution greatly enhanced thereby.

Experiments with comminuted muscle showed that on either side of an isoelectric zone (about pH 4.5 to 6) the addition of HCl to about pH 2, or of NaOH to about pH 10 or 11, occasioned very pronounced swelling. Further addition of acid or alkali caused an apparent decrease in swelling due, presumably, to peptization of the proteins.

NaCl (3 per cent) almost entirely suppressed swelling of comminuted muscle due to HCl below an apparent isoelectric point of pH 4.5. Between pH 4.5 and 7 NaCl caused an increase in swelling greater than that occasioned by alkali alone up to pH 10. Between pH 7 and 8.5 the action of NaCl was about the same as at pH 7, while the addition of NaCl at higher pH values caused an apparent decrease in swelling, probably due to peptization of the proteins.

Above pH about 4.5 the addition of NaCl to comminuted muscle caused a slight but definite decrease in pH, while below this value the pH was increased.

In the pH range examined (5.62 to 7.70) 1 per cent NaCl did not cause appreciable swelling of comminuted muscle. Maximal swelling occurred in 3 to 7 per cent NaCl, depending on the pH, while higher concentrations occasioned an apparent decrease in swelling presumably due to peptization of the proteins.

Comminuted muscle acidified with HCl (pH 3.45) or made slightly alkaline with NaOH (pH 7.10) retained liquid against hydraulic pressure much more markedly than did muscle at its normal pH (6.2). The addition of NaCl (1 per cent) increased the liquid holding power of muscle at its natural pH, or when it was made slightly alkaline, but appeared to almost completely neutralize the liquid-retaining power of acidified muscle.

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Vitamin A and D Potencies of Oil from Body, Liver and Intestines of Pilchard, Herring, Salmon and Tullibee

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ABSTRACT

In vitamin A (colorimetric) and vitamin D (biological) assays on representative seasonal samples of commercially produced pilchard and herring oil (from the whole fish), the vitamin D potency was found to vary inversely with the yield of oil, and the potencies of these oils with laboratory samples of tullibee oil were found to be within the range quoted for authentic cod liver oil, whereas the vitamin A potency was considerably lower than for most fish liver oils. The vitamin D of pilchard, herring, and salmon offal oil was found equally effective for rats and chickens. Vitamin D was found occluded with the stearine fraction of herring oil and was separated from it by three recrystallizations from acetone. The vitamin D potency of herring oil was found not to be related to the size of the fish.

During the last few years considerable interest has been taken in sources of vitamins A and D other than the traditional liver of the codfish. The following factors are of considerable importance in determining the feasibility of utilizing fisheries by-products for their content of vitamins A and D: the amount of material available, the percentage of vitamin-bearing tissues in the fish and their oil content, vitamin potency and seasonal variations, and the effectiveness of the vitamin D of the oil for chickens and for rats. The iodine value, free fatty acid content, percentage of stearine, and unsaponifiable matter are also factors taken into consideration in marketing vitamin oils.

The oil obtained commercially from pilchards (from the whole fish), or the refined oil with the excess stearine removed, has been shown to be an excellent source of vitamin D for poultry (Biely *et al.* 1933-1940) and when blended with the liver oil of other fish may be used as a medicinal oil (Brocklesby and Large 1935). The vitamin A potency of pilchard oil is relatively low when compared to the liver oil of most fish (Milne *et al.* 1937). The vitamin D potency of the oil obtained commercially from herring is found to be within the same range as pilchard oil (Pugsley 1938). No reports have been issued on the vitamin A and D potencies of the oils obtained from the waste material (offal) of the salmon canning industry in Canada. The extensive data reported by Harrison *et al.* (1939) on the offal oil of salmon, caught in the Puget sound and Alaska fishing areas, are undoubtedly applicable to the oil produced in Canada. The oil

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(body oil) obtained from canned sockeye and pink salmon has been shown to have a vitamin D potency within the same range as that of pilchard and herring, but the vitamin A potency of this oil is practically negligible (Bailey 1936). The vitamin A and D potencies of the oil obtained from the tullibee of the mid-western lakes of Canada have not previously been investigated. Thus far this fish has not been utilized for its meal and oil content as has been the case with pilchard, herring, and waste material of salmon. The physical and chemical properties of the oils obtained from the latter material have been very extensively discussed in Bulletin 59 of the Fisheries Research Board of Canada (Brocklesby 1941).

This work was carried out while the author was employed as Assistant Biochemist with the Fisheries Research Board of Canada, at the Fisheries Experimental Station, Prince Rupert, B.C. It was presented to the Canadian Physiological Society in Toronto on November 2, 1940.

ORIGIN OF MATERIAL

The samples of pilchard (*Sardinops caerulea*) and herring (*Clupea pallasii*) oils were obtained from reduction plants along the British Columbia coast. Arrangements were made with the foremen of the plants to submit weekly samples of oil throughout the fishing season. The individual samples represented the oil obtained from several tons (>100) of fish (1 ton equals 907 kg.) For pilchard oil, data on the yield of oil in U.S. gallons (1 gal. equals 3.8 litres) per ton of fish reduced were also collected. From the whole group of samples submitted, representative samples were chosen showing significant differences in yield, and vitamin D assays were carried out on these.

Samples of the whole fish in the case of the pilchard and herring were obtained from catches landed at Nootka and Prince Rupert, B.C., respectively. "Tullibee" were obtained from lake Winnipeg, Man., and Lac la Bische, Alta., and "lake herring" from Lesser Slave lake, Alta. Dr. A. L. Pritchard and Dr. W. A. Clemens of the Pacific Biological Station, Nanaimo, B.C., have kindly examined these, and identified them as being all *Leucichthys tullibee*. The data for the Pacific salmon—sockeye (*Oncorhynchus nerka*), pink (*O. gorbuscha*), chum (*O. keta*), spring (*O. tschawytscha*) and steelhead (*Salmo gairdneri*)—were obtained from material (offal) procured from fish landed at Sunnyside Cannery on the Skeena river, B.C.

METHODS

Vitamin A potency was determined by the photocolorimetric method of Dann and Evelyn (1938). The results are expressed in blue units per g., since this notation has been used more frequently than the term L value proposed by the above authors. The L value per mg. was multiplied by 500 to convert it to blue units per g. The estimations were made on the unsaponifiable fraction of all samples of oil, in which provisional readings showed a value below 10,000 blue units per g. The procedure outlined by Hume and Chick (1935) for separation of the unsaponifiable fraction was followed. The number of blue units per g. multiplied by 1.7 ± 0.3 gives an approximate valuation in terms of

international units (Pugsley 1939a). The treatment of the material for the estimation of vitamin A in the various organs of the fish was carried out by the method outlined by Pugsley (1939c). The vitamin D assays were done according to the technique adopted by the U.S. Pharmacopoeia 1934 Revision, and the results were calculated according to Coward's method (1938).

AVAILABILITY AND COMMERCIAL PRODUCTION

The Bureau of Statistics (1940) reported the following values for weight of fish landed and gallons of oil produced during 1938 (1 gal. equals 4.55 litres): pilchard, 51,768 tons and 2,195,850 gallons; Pacific herring, 66,446 tons and 929,158 gallons; Pacific salmon, 86,733 tons and 114,797 gallons; tullibee (Prairie provinces), 2,517 tons and no oil. It is noted that over three million gallons of oil were produced in 1938 from herring, pilchard, and salmon in British Columbia and the production for 1936 to 1939 shows that from about one and a half to three million gallons of these oils are obtained annually. The production of salmon oil is relatively low because only a few companies utilize the offal for meal and oil. The waste in the salmon canning industry is approximately 25 to 35 per cent of the weight of the fish; this material yields 20 to 40 U.S. gallons of oil per ton, depending on the species of salmon and time of reduction. If all this waste material could be utilized for meal and oil, the annual production of salmon oil noted above would be increased five to seven times.

RESULTS

Data on the percentage of total abdominal viscera (exclusive of gonads), liver, and intestines (portion of the gastro-intestinal tract distal to the stomach including mesentery, spleen, pancreas, etc.), and on the percentage of oil in the body and these organs of pilchard, herring, and tullibee are presented in table I. Circumstances did not permit collection of the complete data for the salmon. The values are the means of single samples of fifty or more fish in each case taken on one date during the fishing season and hence do not represent seasonal variations. The percentage of liver and intestines in these fish is

TABLE I. Percentage of total viscera, liver, and intestines and percentage of oil in these organs of pilchard, herring, tullibee, and Pacific salmon

Date of catching	Kind of fish	Mean weight (kg.)	Total viscera in fish (%)	Liver in fish (%)	Intestines in fish (%)	Oil in fish (%)	Oil in liver (%)	Oil in intestines (%)
28/8/37	Pilchard	0.20	7.0	3.0	3.2	15.6	6.8	8.0
16/3/38	Herring	0.13	3.9	1.0	1.2	7.3	3.1	12.0
28/2/39	Tullibee	0.43	5.3	1.2	3.3	8.2	6.8	6.3
29/7/38	Sockeye	3.22	...	1.2	...	...	6.5	6.5
4/8/38	Pink	1.82	...	2.3	...	...	4.3	3.5
4/8/38	Chum	4.54	...	2.1	...	...	4.2	2.3
2/8/38	Spring	11.40	...	2.0	...	...	5.0	3.0
2/8/38	Steelhead	3.22	4.2	1.3	2.4	...	15.2	44.7

within the range reported for halibut (Pugsley 1939b) and Pacific cods (Pugsley 1940). The percentage of oil in the livers (except steelhead salmon) is relatively low when compared to halibut and cod, while the percentage of oil in the intestines is within the same range (Pugsley 1939b, 1940). It is noted that pilchard contain approximately twice as much oil as herring. Because of the low oil content of the liver, it is doubtful if it would be economical to process the livers of these fish for their oil and vitamin content.

The data obtained on the distribution of vitamins A and D in the whole fish (exclusive of viscera, liver, and gonads), liver, and intestines separately, are presented in table II. The data on the vitamin D potency of the salmon body and visceral oils were not obtained. The body oil of these fish did not give a measurable amount of vitamin A by the Carr-Price test. In most cases the liver oil contains more vitamin A than the visceral oils. The vitamin D potency of the liver oils is higher than is usually reported for cod liver oil, but is not as high as for halibut or Pacific cods. Harrison *et al.* (1939) report 20 and 90 international units for the visceral oil of steelhead and spring salmon respectively, and Bailey (1936) found 50 to 70 international units of vitamin D in the oil of canned sockeye and pink salmon. The vitamin D potency of the oils obtained from the body and intestines separately of the remaining fish has not yet been determined.

TABLE II. Distribution of vitamins A and D in oil from body, liver, and intestines of pilchard, herring, tullibee, and Pacific salmon

Kind of fish	Body oil		Liver oil		Intestinal oil
	Vitamin A (blue units per g.)	Vitamin D (inter. units per g.)	Vitamin A (blue units per g.)	Vitamin D (inter. units per g.)	Vitamin A (blue units per g.)
Pilchard.....	0	54	38000	200	....
Herring.....	0	33	22500	250	1000
Tullibee.....	0	90	30000	...	8000
Sockeye.....	...	...	12000	300	12000
Pink.....	...	...	4000	200	4900
Chum.....	...	...	3000	200	300
Steelhead.....	...	...	4300	200	1100
Spring.....	...	...	13000	125	8000

The vitamin A values of commercially produced pilchard oil (table III) are quite variable throughout the year and do not show any definite trend with season or yield; on the other hand there is a highly significant inverse relationship between the yield of oil and its vitamin D potency as shown in figure I. At the beginning of the fishing season in late July, when the yield is from 30 to 40 gallons per ton of fish, the vitamin D potency is between 90 and 100 international units. As the fishing season advances, the yield increases and the vitamin D potency decreases to values between 35 and 50 international units. The vitamin A potency of this oil is relatively low when compared to most fish

TABLE III. Seasonal variations in potency of vitamins A and D and yield of oil from pilchard reduced at Kildonan, B.C. (1 U.S. gal. equals 3.8 litres; 1 ton equals 907 kg.)

Date of production	Vitamin A (blue units per g.)	Vitamin D (international units per g.)	Yield (U.S. gal. per ton)
1/8/37	150	95	30
17/8/37	240	50	51
2/9/37	60	35	56
4/10/37	220	70	50
28/12/36	180	50	50
30/7/38	125	100	40
28/8/38	250	65	51
16/9/38	260	55	55
2/10/38	230	50	60

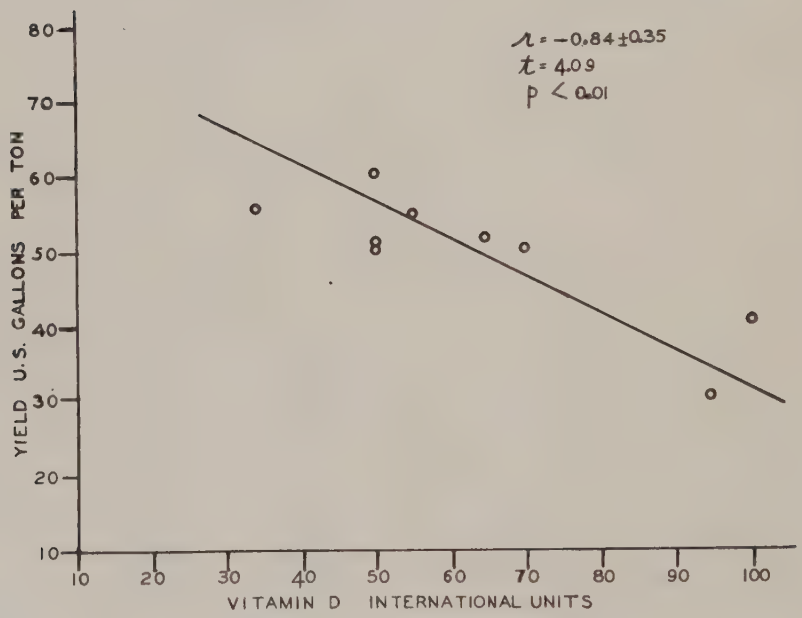

FIGURE I. Inverse relationship between yield and vitamin D potency of pilchard oil

liver oils. As indicated in table II, practically all the vitamin A is contributed to these oils from the liver and intestines. Owing to the small size and low oil content of these organs, in comparison with the remainder of the fish, the amount of vitamin A in the oil produced from the whole fish is necessarily relatively low, because of the dilution factor. Also, the methods of production are not usually carried out in a manner to conserve vitamin A.

Data on the yield of commercially produced herring oil for samples showing seasonal variations in potency of vitamins A and D (table IV) were not available

at the time the assays were carried out; however, data on the yield of subsequent samples collected in 1938-1939 are submitted to show the usual variations encountered. It is seen that vitamin A potency of this oil tends to be less than that of pilchard oil, while the vitamin D potency is within the same range. The yield of oil is lower than that of pilchard and a similar inverse relationship is indicated between the yield and vitamin D potency; calculating from the data available $r = 0.53 \pm 0.33$, $t = 1.42$, $p = < 0.2$. As the fishing season advances from October to March, the yield gradually decreases, and the vitamin D potency increases. The sample of oil obtained in June was from the first catch of herring obtained during the summer fishing season. The yield at this time is relatively high and the vitamin D potency low.

TABLE IV. Seasonal variations in vitamin A and D potencies and yield of herring oil (U.S. gal. equals 3.8 litre; 1 ton equals 907 kg.)

Date of production	Locality of catching	Vitamin A (blue units per g.)	Vitamin D (inter. units per g.)	Date of production	Locality of catching	Yield (U.S. gal. per ton)
15/10/36	Barclay sound	25	30	21/11/38	Barclay sound	38
15/10/36	Esperanza inlet	20	50	28/11/38	Prince Rupert	30
15/10/36	Cousins inlet	76	50	2/12/38	Barclay sound	33
15/11/36	Esperanza inlet	26	65	19/12/38	Prince Rupert	23
27/11/36	Cousins inlet	30	75	9/ 1/39	Prince Rupert	20
14/12/36	Esperanza inlet	28	35	24/ 1/39	Namu	17
23/ 1/37	Barclay sound	30	50	31/ 1/39	Prince Rupert	19
8/ 3/37	Prince Rupert	40	50	28/ 2/39	Surf inlet	5
8/ 3/37	Prince Rupert	20	75	5/ 3/39	Laredo inlet	8
27/ 6/38	Zayas island	60	36	27/ 6/38	Zayas island	42

The vitamin D potency obtained in both rat and chick assays of the same samples of pilchard, herring, and salmon offal oil (table V) agrees within the limits of error of the assay, and it may be concluded that the vitamin D of these oils is equally effective for rats and chickens.

TABLE V. Rat and chick assays of vitamin D potency of herring, pilchard, and salmon oil

Oil	Rat assay (international units per g.)	Chick assay (A.O.A.C. chick units per g.)
Pilchard N.H.B. 7.....	50	55
Herring C.52.....	38	33
Herring P.E. 14.....	60	56
Salmon offal oil P.C.S. 39..... (From 75% Pink, 15% Chum, and 10% Sockeye offal)	130	135

For oil procured from tullibee under laboratory conditions (table VI) there is a marked difference in the percentage from fish from the different localities. The high yield from the fish obtained from lake Winnipeg is similar to the value obtained from pilchard and herring. The potency of vitamins A and D, and the iodine value, are within the range found for pilchard oil, whereas the percentage of unsaponifiable matter in this oil is somewhat higher than is usually reported for herring and pilchard oil.

TABLE VI. Yield, vitamin A and D potencies, iodine value, and percentage of unsaponifiable matter of tullibee oil

Date of production	Locality of catching	Oil (%)	Vitamin A (blue units per g.)	Vitamin D (inter. units per g.)	Iodine value	Unsaponifiable matter (%)
22/2/39	Lesser Slave lake	4.8	260	75	142	1.94
28/2/39	Lake Winnipeg	16.2	100	50	169	1.38
23/3/39	Lac la Bische	2.8	230	150	...	....

The relatively high stearine content of pilchard and herring oil has been an obstacle to their acceptance as a vitamin supplement. At present there is no method of measuring accurately the amount of this substance in an oil. The usual method of removing stearine consists in cooling the oil to 0° C., or lower, for several days and then passing it through a filter press. Naturally, a considerable amount of oil is occluded with the stearine separated by this method. Brocklesby and Denstedt (1930) found the solid portion (stearine fraction) of crude pilchard oil to have a higher vitamin D potency than the cleared oil (stearine-free). It seemed of interest to see to what extent vitamin D was bound to the stearine fraction, by use of a method that permits recrystallization and washing of the separated stearine.

In order to do this, advantage was taken of the fact that the saturated glycerides of the oil are more insoluble than the unsaturated glycerides in cold acetone. To 200 g. of crude herring oil, assaying 60 international units per g., 320 ml. of acetone was added, and the mixture cooled in an ice bath for three hours. The precipitated stearine was filtered off and washed with cold acetone. The excess acetone was removed from the precipitated stearine by placing *in vacuo* for 12 hours at 40° C. This fraction was assayed for vitamin D and found to contain 23 international units. The yield of stearine by the above method was 4.3 per cent. In order to see whether further purification of the stearine would remove the vitamin D, another sample of the original oil was taken, and the stearine separated as above and recrystallized three times from acetone. The resulting stearine fraction did not give any evidence of healing of rickets when fed at a 25 international unit level, which indicates that it was practically free of vitamin D. These results show that vitamin D can be separated from the saturated glycerides by the above method, although its presence in the first fraction shows it to be quite firmly occluded with the stearine.

In view of the variations in the vitamin D potency of herring oil, and the

fact that the vitamin A potency of the liver oil of halibut and grayfish (Pugsley 1939a, b) are related to the size or age of the fish, it appeared of interest to determine the vitamin D potency of herring oil as related to the size of the fish. In order to test this supposition, 150 lb. (6.8 kg.) of herring was obtained February 21, 1938, and divided into three groups of fish of the following lengths: 6 to 7, 7 to 8, and 8 to 9 in. (6 in. = 15.2 cm.), respectively. The oil was obtained from each group separately by cooking in a retort, pressing the cooked material, and centrifuging the press liquor. The samples assayed 170, 130, and 145 international units for the above groups, respectively. These results indicate that the oil from the smaller fish has a higher vitamin D potency than that from the larger fish, but the differences are of doubtful significance. Possibly variations in oil content of the different groups played a more important role in determining the vitamin D potency of the oil than the size of the fish. The former is indicated as a factor for determining the vitamin D potency of the commercial samples listed in table IV. The treatment of these groups of fish did not permit an accurate determination of the percentage of oil in the fish.

DISCUSSION

Owing to the low oil content and relatively small size of the livers of pilchard and herring, it is not practicable under the present methods used in fish oil plants to separate and extract these livers for their vitamin content. The more practicable method is to utilize the whole fish for its meal and oil content. The addition of the viscera of fish utilized in the smoking, salting, and canning industry to the fish processed in reduction plants is certainly justified, as practically all the vitamin A contained in the oils produced commercially from whole fish is contributed to them from these organs. The negligible amount of vitamin A in the body tissues of fish is a point not always recognized by dietitians and companies advertising fisheries products. Edisbury *et al.* (1937) have reported that eels contain significant amounts of vitamin A in their body tissues, but, in general, the body tissues of salt water fish are very low in this constituent.

The vitamin D potency of the pilchard and herring oil produced commercially in British Columbia is within the range quoted by most workers for authentic cod liver oil, as produced in Newfoundland and Norway (MacPherson 1937). The vitamin A potency of these oils is relatively low, for the reason pointed out above, and hence it is necessary to fortify them with this constituent in order to compete with cod liver oil. The oils extracted from intestines of halibut and Pacific coast cods are well suited for this purpose, as the vitamin D potency of the latter is relatively low (100 to 500 international units) and the vitamin A potency relatively high (100,000 to 200,000 international units) (Pugsley 1939b). Pilchard and herring oils are well adapted for use as base oils for fish liver oils with such high vitamin A and D contents as swordfish, tuna, etc. If the occasion should arise to process tullibee for their meal and oil content, the vitamin A and D values reported here show that this oil would be similar in its vitamin potency to herring and pilchard oil.

The utilization of the large amount of waste material from the canning of salmon in British Columbia has been a problem that has confronted the industry

for some time. A relatively large amount of oil (15 to 20 per cent) may be obtained from the heads and fins of salmon. This oil is similar to pilchard and herring oil in its vitamin D potency (Harrison *et al.* 1939). When the viscera of salmon are added to the heads and fins, and the whole processed, the vitamin A and D potency of the resulting oil is within the upper range reported for authentic cod liver oil. Salmon offal oil usually contains considerable red pigment, and for this reason has not been generally accepted as a vitamin supplement. There is no evidence that this pigment is deleterious and it has been reported to consist chiefly of asticin-like compounds (Bailey 1937). The low oil content of the liver of salmon (except steelhead) has not made it practicable to process these livers with present-day methods for their vitamin A and D content. Steelhead salmon are not used very extensively in the canning industry, and are usually marketed as the whole fish, with viscera intact. This practice does not lend itself to a practical method of collecting and processing the by-products of this fish. If this practice should change, the results obtained here indicate that these by-products are worth processing for their vitamin content.

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Studies on Salt Fish

VII. Red Halophilic Bacteria in Seawater and Fish Slime and Intestines

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ABSTRACT

Twenty-four samples of seawater, fish slime and intestinal contents, taken off Halifax harbour, yielded two red halophilic sarcina strains from codfish slime.

Red halophilic bacteria are always associated with solar salt and the salterns in which the seawater is allowed to evaporate. Harrison and Kennedy (1922) examined brines of various strengths from Turks Island reservoirs and found red halophiles in those of 65 to 100 per cent salinity but none in weaker brines when cultured in salt codfish broth and agar or sterilized salt fish. On the other hand, additions of sterile Irish moss to flasks of brine resulted in growth of red bacteria even in the weaker brines and seawater after 10 days at 37°C. They concluded that "seaweed and seawater are sufficient nutriment for vigorous growth of the red organisms and in all probability this microbe (*Pseudomonas Salinaria*) is a seawater organism, which can adapt itself to growth in strong brine, and can resist for a considerable period desiccation brought about by evaporation of seawater under a tropical sun accompanied by warm winds".

The Research Department of the Salt Union, Liverpool, England (personal communication) examined numerous seawater samples from many parts of the world (north and south Atlantic shipping routes, shipping route from Liverpool to Singapore via Suez) and found invariably a flora of halophilic organisms, very frequently yellow, orange or red, or all together, when the samples were cultured in suitable saline media. Stuart (1938) showed that red halophilic bacteria are widely distributed in nature, for example, water in stagnant pools, sulphur springs, soil, dung, freshly flayed calf skin, and can be cultivated from sources other than salt or concentrated brines if allowance is made for exceedingly long lag periods.

Because of the losses resulting from the growth of these organisms on salt fish, an attempt has been made to determine, in so far as the Canadian Maritimes are concerned, if the primary contamination is entirely from solar salt, or if the organisms can be found in the bacterial flora of our seawater or of the fish slime or intestines.

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In the present tests, Stuart's (1938) technique was followed, inoculating the samples of seawater, fish slime and intestinal contents under aseptic precautions directly into large test tubes containing 25 ml. of sterile nutrient brine consisting of 25 per cent c. p. sodium chloride, 1 per cent peptone, 0.5 per cent calcium chloride and 0.5 per cent magnesium sulphate ($MgSO_4.7H_2O$) in distilled water. One ml. portions of the inoculated and uninoculated control tubes were withdrawn before and after incubation at 37°C. for 30, 60 and 90 days and plated on a modified Lochhead's (1934) skim-milk salt agar (25 per cent sodium chloride). The plates were then incubated at 37°C. for 30 days and all growths recorded.

On four different occasions (October 1938 to September 1939) the following total numbers of samples were taken near the outer entrance to Halifax harbour: seawater at the surface and at depths to 30 m., 11 samples; fish slime, 7 samples; fish intestinal contents, 6 samples. Red halophilic bacteria could not be isolated from any of the seawater samples or the samples of intestinal contents, but the slime from two different codfish, sampled before the fish were taken into the boat, yielded cultures of red halophilic sarcinae after incubation for 30 and 60 days respectively in the nutrient brine described above.

No growth of red organisms was obtained when Irish moss (*Chondrus crispus*) was added to the seawater in culture dishes in which a gypsum block had been placed following the technique of Harrison and Kennedy (1922).

Obviously the number of samples is too small and the locality and season are too limited to draw any general conclusions, but the potential presence of red halophilic bacteria in these habitats points to their danger as a source of contamination of salt fish unless the latter are treated to prevent the growth of such organisms on them.

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Regeneration of the Scales of Atlantic Salmon

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ABSTRACT

On removal of scales in the smolt new scales develop in the scale pockets, attaining normal width in 40 days, but not quite normal length even by 59 days. Initial growth is very rapid and without circulus formation, but with decreasing rate circuli are formed with intervening spaces diminishing in width and there is often a check when normal growth commences. Scales near a large area of regeneration show checks corresponding to time of most rapid regeneration. Pairs of small and large regenerating scales occur, each pair apparently developing from the one pocket.

Normally the scales of salmon remain on the fish throughout life and show annual checks indicative of the age of the fish. However, scales are easily removed from their pockets and when this happens new scales grow in the same pockets, replacing the old scales. These replacement or regenerated scales are usually readily recognized because the ridgeless central area of the scales is abnormally large. They are also abnormal because they do not show the true age of the fish but only the age subsequent to the time of scale removal.

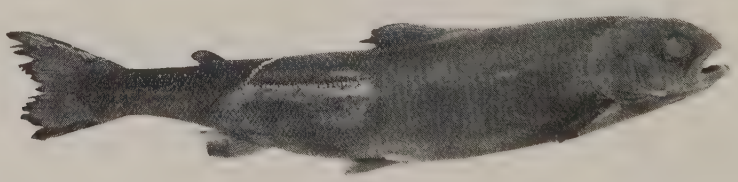

Figure 1. Salmon smolt showing ventro-dorsal row of guide mark scales and the antero-posterior row from which every other scale was successively removed (table I).

MATERIAL

During the summer of 1936 an experiment was carried out on a salmon smolt to determine the rate of scale regeneration. Additional information on scale regeneration was procured from other experimental fish and from the 1932 run of salmon taken by drift nets on the coast of St. John county, New Brunswick.

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RATE OF REGENERATION

On July 17 several scales were removed from a row running dorsad from the lateral line on the right side of a smolt near the adipose fin (fig. 1), the row of empty pockets thus providing for a time a guide mark for the removal of other scales. From the third antero-posterior row above the lateral line the fifth scale anterior to the guide mark was also removed on the same date. Proceeding anteriorly scales were removed from this row at intervals as shown in table I, and the fish was finally killed, so that the new scales replacing those removed were successively younger at the time of death.

TABLE I. Date of removal and age and number of circuli of new scale (by September 14, when the fish was killed) for every other scale from antero-posterior row of right side of smolt. New scale no. 25 was not found, presumably because it was so small.

Scale no.	5	7	9	11	13	15	17	19	21	23	25
	July		August					September			
Removal date	17	26	3	10	17	23	28	1	4	6	7
Age (days)	59	50	42	35	28	22	17	13	10	8	7
Circuli (no.)	8	7	5	6	4	3	2	0	0	0	?

Numbers 1, 5 and 7 of the new scales were fifty days or more old (table I) and appear of normal size (fig. 2), but the others were less than normal and graded in size with the length of the period of regeneration, scale no. 25 not being visible and no. 23 being barely exposed.

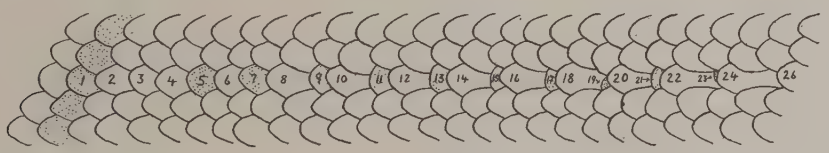

FIGURE 2. Sketch showing regenerating scales (stippled) of different ages (table I) at end of experiment in relation to surrounding normal scales.

Since there is considerable variation in the sizes of scales from different regions of the body of a fish (Esdaile 1912), it is desirable to compare these new scales with the original scales and with the corresponding scales of the other side of the body (fig. 3). The length (fig. 3, above) of the new regenerating scale evidently increases very rapidly for the first 17 days but considerably less from the 17th to the 59th day. At 50 days the size of the original scale is reached and subsequently it is exceeded, thus indicating growth of the fish in the interval. Actually the fish increased in length from 226 to 276 mm., an increase of 22 per cent. Even by 59 days the regenerated scale had not reached the length of the normal scale of the other side, but no data are available as to the length of time that would be required for this. It will be seen in figure 3, below, that the width

of the regenerating scale reaches that of the original scale in about 35 days and that of the normal scale in 40 days.

The exposed posterior portion of regenerated scales is quite often much smaller than in normal scales and less frequently is distorted from the main axis (fig. 4A). Possibly this area of the scale is retarded in growth by the injury to

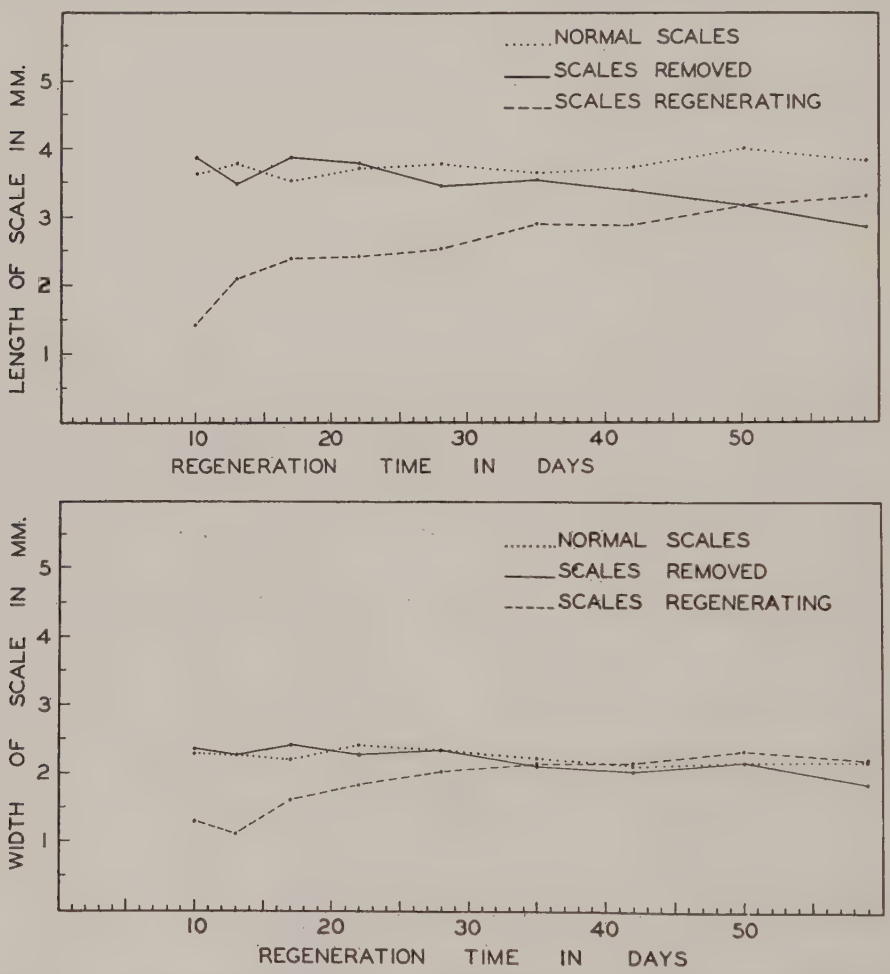

FIGURE 3. Above: Lengths of the new regenerating scales in relation to age, with for comparison the lengths of the original scales removed from the same pockets and of the corresponding normal scales of the other side. Below: Widths of the new regenerating scales in relation to age, with for comparison the lengths of the original scales removed from the same pockets and of the corresponding normal scales of the other side.

the scale pocket and, if so, this would account for the more rapid regeneration of the scale to normal size in width than in length.

FORMATION OF SURFACE SCULPTURE

For the first 13 days, when the length increased most rapidly (fig. 3 above)

there were as yet no circuli laid down on the scale (table I and fig. 4B), but from the 17th to the 59th day, when the length increased more slowly, the number of circuli increased gradually from 2 to 8 (4 to 5 circuli after 28 days, fig. 4C).

The spacing of the circuli is similarly determined by the growth of the scale. The first circuli that are laid down when the scale is growing rapidly are quite far apart, but the circuli become progressively closer together as scale growth decreases when normal size is approached. A check is often formed on regenerating scales when they completely fill the pocket and commence normal growth (fig. 4D). The nature of the check varies considerably, presumably depending on the rate of growth of the fish at the time. In some cases there are two or more circuli very closely approximated, while in other instances the spacing of the circuli grades more or less insensibly into the normal. Such a check might be used to determine the size of the fish when a particular injury occurred.

Although the large focal area of regenerating scales does not possess any circuli, it does have small elevations scattered irregularly over the outer surface. They give this part of the scale a granular appearance and can easily be felt with a fine probe. It would seem then that the cells concerned with circulus formation are irregularly arranged when the regenerating scale is first laid down and that they do not become regularly arranged around the periphery of the scale to form circuli until the rate of growth slows down somewhat as shown above. Moreover the first few circuli that are laid down are very wavy and irregular (fig. 4D), which is no doubt due to the irregular outline of the young regenerating scale which is quite marked in figure 4B, a scale that has been regenerating for 10 days. This suggests that the forking of circuli so often found in normal scales is probably the result of an increased growth rate of that particular part of the scale.

Since the osteoblasts, which produce the outer layer of the scale, are found normally only at the periphery of the pocket, regeneration of a new scale in the centre of the pocket would necessitate either the migration of the osteoblasts to this area or their formation from other cells. Concerning this, Neave (1936) states: "It seems likely that the follicle cells play an important part in regenerating scales, for removal of a scale involves also the loss of all or most of the osteoblasts."

Backman (1932) rejects the interpretation of abnormal scales with large foci as being those formed to replace scales which have been lost. From one salmon he examined 113 scales from the side and belly of the fish, of which 53.1%, 30.9% and 11.5% were laid down in the 1st, 2nd and 3rd years respectively in the river and 1.8%, 1.8% and 0.9% respectively in the 1st, 2nd and 3rd years in the sea. He believes that these proportions show that the abnormal scales cannot be regenerated scales but must be new scales intercalated between older ones. However, such abnormal scales have been experimentally produced by regeneration in *Fundulus heteroclitus* by Scott (1912) and in the bluegill (*Helioperca incisor*) by Creaser (1926) as well as in the experiment described. Also Backman's results do not show that such abnormal scales are late-appearing intercalated scales and he makes no attempt to distinguish between a regenerated scale and a newly formed intercalated scale. Moreover he states that the circuli surrounding the focal area of these abnormal scales are more widely spaced than in normal scales, which

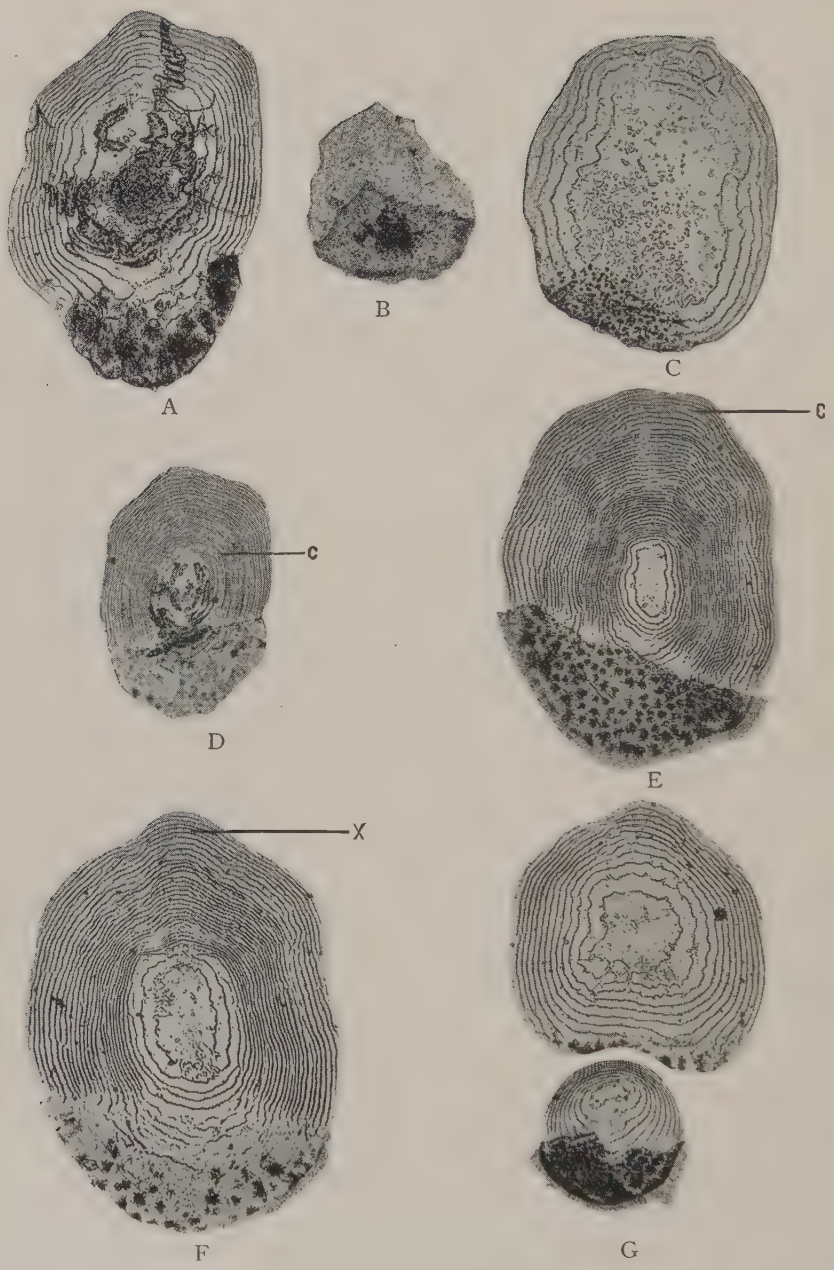

FIGURE 4. A: Regenerating scale showing small size and distortion from the main axis of the exposed posterior portion. B: Regenerating scale (no. 21, 10 days old) without circuli. C: Regenerating scale (no. 13, 28 days old) with 4 to 5 circuli. D: Regenerated scale showing large focus followed by wide circuli formed during regeneration and then narrow circuli (c) when normal growth commences. E: Normal scale adjacent to regenerating scales, showing check (c) formed during their rapid growth. F: Normal scale as in E, but from 9th pocket posterior to regenerating scales and not showing any check (x) during their rapid growth. G: Pair of large and small regenerated scales of same age and apparently replacing one lost scale; the small scale was in a separate pocket beneath the large one with only the dark area exposed.

is the case with regenerated scales, and makes it quite probable that he was merely dealing with regenerated scales.

EFFECT OF REGENERATION ON ADJACENT SCALES

Ten scales on the right side of a 263 mm. smolt opposite the posterior base of the dorsal fin and immediately above the lateral line were removed from their pockets and put back in place again, but did not remain and new scales were regenerated. The normal scales adjacent to the regenerated scales were found to have checks in the form of 1 or 2 narrow, discontinuous circuli (fig. 4E, a normal scale immediately anterior to 4 regenerating scales in the same row). The check is followed by several wide circuli so it is quite clear that growth was retarded mainly or perhaps only during the early stages of regeneration when growth is most rapid. In scales more distant from the area of regeneration (fig. 4F) the circuli (five) at the edge of the scale, that are narrower than the preceding circuli and were laid down during the experiment, are not preceded by any definite check, which indicates that the growth of these scales was not retarded during the rapid growth of the regenerating scales. Normal scales surrounding single regenerating scales fail to show any appreciable check, so apparently the definiteness of the check on normal scales depends on the number of scales which are being regenerated in that vicinity, that is, the check is caused by the neighbouring regeneration.

In twelve experimental fish, about 50 scales were removed from a definite region on each at the beginning of the experiment, and at the end of the experiment the normal scales at various distances from this region were examined for checks due to scale regeneration. In ten of the fish the normal scales irrespective of distance from the area of regeneration possessed at the place where should appear, if present, the checks due to regeneration, checks that appear to have been due to handling. These checks confused the issue considerably; nevertheless the checks on scales near the area of regeneration did appear to be more definite than those on more distant scales. The two remaining fish, however, which had been anaesthetized and thus had struggled less in handling did not show in the scales generally a check due to handling, but merely the transition to narrower circuli during the experiment, and the scales near the area of regeneration showed fairly definite checks in the form of 2 or 3 discontinuous, narrow circuli. This check was not found more than 3 scales distant from the regenerating scales. The retarding effect of regenerating scales on normal scales thus seems to be quite local.

Absorption of part of the scale was also noticed in normal scales surrounding regenerated scales in practically all the fish examined, but to a varying extent. The degree of absorption varied considerably, but was more commonly slight. The absorption occurred at one or several points anywhere on the periphery and in extreme cases the entire edge was absorbed. Since the absorption of normal scales coincides with the time of regeneration, it might be thought that the normal scales are absorbed to supply material for rapid growth of the regenerating scales, but the haphazard arrangement in relation to the area of regeneration of scales showing absorption is against such a view. It is believed that the scales are

absorbed because they were displaced in their pockets. Owing to the overlapping of the scales and the handling of the fish it would be quite difficult to remove a large patch of scales without disturbing surrounding scales. That displacement of scales results in absorption of part or all of the edge of a scale was found in an experiment to be reported elsewhere.

SMALL REGENERATED SCALES

Although most of the regenerating scales eventually reach the size of normal scales, some of them remain small. In 5 out of 15 experimental fish examined, from 1 to 21 small but apparently fully regenerated scales were found in the area of regeneration comprising about one square centimetre. Except for size the majority of these small scales look exactly like the large regenerated scales, but some are distorted into queer shapes, while others appear to be parts of a scale. The small scales invariably possessed a few circuli less than the large regenerated scales.

The occurrence of small regenerated scales was puzzling until the large regenerated scales were removed individually, when in five cases a small scale was found beneath the posterior end of a large one and completely covered by it except for the posterior tip. The numbers of circuli in the small and large scales (fig. 4G) respectively were: 4, 7; 4, 6; 5, 8; 4, 5; and 9, 11. The posterior ends of the large scales were somewhat stunted, for, while the posterior end of a normal scale is rounded, in two of the large scales it was truncate and in the other three it was indented where the small scale was situated (fig. 4G). The amount of overlapping when the scales were in situ is indicated by the intact epidermis on the small scale. The small scales were situated in separate pockets directly beneath the large scales. For this reason we can be reasonably sure that the small scales are additional scales and not merely the result of crowding by adjacent scales. Also, normally one scale does not directly overlap only one scale behind it but the adjacent edges of two scales.

It might be assumed in these cases that, when the scale was removed, two regeneration centres were set up in the same pocket, one anterior and the other posterior, that regeneration began earlier or proceeded more rapidly in the anterior regeneration centre and therefore produced the larger scale, and that the posterior one grew in under the anterior one with formation of a second pocket. Double regenerated scales have also been noticed, evidently formed by the early fusion of two such scales.

Not only have these small scales been found in experimental fish of smolt size, but also in adult salmon of the St. John river taken in 1932 a fair number with small regenerated scales were noted. In these, regeneration had occurred at various times,—in the second summer in the river, in the smolt stage, in the first summer in the sea, in the second summer in the sea and after the second winter in the sea. In each case the time was determined by comparing the years of growth on the regenerated scales with the years of growth on normal scales. The small regenerated scales show the growth (subsequent to regeneration) as used in age determination, but the annual checks are not as distinct as in the large regenerated scales.

SUMMARY

In an experiment on a salmon smolt regenerating scales were found to reach in about 35 days the width of the original scale at time of removal from the same pocket and the width of contemporaneous normal scales in 40 days. They did not reach the length of the original scale until 50 days and even by 59 days had not reached the length of normal scales.

The initial growth of regenerating scales is very rapid and during this time circuli are not formed, but, as the rate of growth decreases, circulus formation begins, the circuli first formed being widely separated. The forming circuli are progressively closer together as the scale approaches normal size, and a check is often formed when it fills the pocket and commences normal growth.

Normal scales adjacent to an area containing a large number of regenerating scales form a check at the time of most rapid growth of the regenerating scales. The effect on normal scales is not apparent when only a few scales have been regenerated, and when about fifty scales have been regenerated it does not extend beyond three scales from the regenerating area. Some normal scales surrounding regenerating scales undergo some absorption at the time of scale regeneration, but this is considered as due to scale displacement rather than as the effect of the regenerating scales.

In areas of scale regeneration in experimental fish several examples were found of a small regenerated scale being situated in a pocket directly beneath the posterior part of a large regenerated scale of the same age, and evidently representing an additional scale. Similar regenerated scales much smaller than normal were also found in adult salmon from off the Saint John coast. They had been regenerated at various ages in the river and sea life of the fish and showed the growth (subsequent to regeneration) as used in age determination.

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Food of Rainbow, Cutthroat and Brown Trout in the Cowichan River System, B.C.

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ABSTRACT

Stomach contents of 293 rainbow, 160 cutthroat and 113 brown trout were examined and analyzed according to species, size groups (5 cm.) and habitat (river or lake). For rainbows of both river and lake (4 to 50 cm. in length), insects, chiefly Trichoptera and Simuliidae, were predominant in all size groups. Fish constituted a small fraction of the food except in the winter when salmon eggs were available. River cutthroat subsisted chiefly on insects (Trichoptera) up to 15 cm., on insects and fish (*Gasterosteus*) up to 30 cm., thereafter largely on fish. Lake cutthroat samples did not eat fish in any number until 41 cm. long. Brown trout ate chiefly insects (Trichoptera) up to 45.5 cm., thereafter turning more definitely to a fish (salmonid) diet. The cutthroat were more piscivorous than the brown trout. A definite selection of food by the trout was indicated.

As part of the biological investigation of the Cowichan river system on Vancouver island, being carried out by the Fisheries Research Board of Canada, a study of the food of the fishes of the system is being conducted. In any fisheries conservation problem a primary requisite is a thorough knowledge of the food requirements of the fish involved. The problem of what the trout eat is of particular significance in the Cowichan system by virtue of the recent introduction of the brown trout (*Salmo trutta* L.) into waters normally inhabited by the rainbow (*S. gairdneri* Rich.) and the cutthroat (*S. clarkii* Gibb.). The study of the food of the brown trout indicates the general effect of this species' association with the others, the rainbow and cutthroat, whether through predation or competition.

MATERIAL AND METHODS

The stomachs of 592 trout were collected between May, 1937, and September, 1939. Most of these were obtained in 1937 and 1938, and all but a few in the months of May to September, inclusive.

Sixty-seven stomachs contained no food. These included 23 rainbow, 15 cutthroat and 29 brown trout. The main study is based, therefore, on the contents of 258 rainbow (226 from the river and 32 from the lake), 156 cutthroat (124 from the river and 32 from the lake), and 111 brown trout (all from the river). In addition, a small collection of stomachs of trout caught during the winter months of 1940 and 1941 has been made available. This includes 35 rainbow, 4 cutthroat and 2 brown.

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All of the larger "river" fish were captured in the upper reaches of the Cowichan, most of them within one mile (1.61 km.) of the source, Cowichan lake. The fry and fingerlings were captured in Oliver and Beadnell creeks, small streams tributary to the Cowichan river near its source and in the Robertson river, which flows into the lake. The "lake" fish were captured at various points in Cowichan lake, mostly in the south arm.

The rainbow trout ranged in length from 4 to 50 cm. (1 1/2 to 19 3/4 in.), the cutthroat from 3 to 59.5 cm. (1 3/16 to 23 1/2 in.) and the brown from 2.5 to 59.5 cm. (1 1/16 to 23 1/2 in.). Most of them were obtained by gill-net but a considerable number were received through the co-operation of anglers and residents of lake Cowichan, whose aid is much appreciated.

In removing the digestive tract the intestine was snipped off just below the stomach. No effort was made to identify the intestinal contents, since undue emphasis would thus be placed on the hard-shelled and protected forms. No attempt was made to make specific identifications of the organisms eaten except in the case of the fishes.

The main criterion on which the relative importance of any organism is based is the number of stomachs which contain this particular organism. To some extent this method may distort the picture since a small number of one form might occur in all the stomachs while large numbers of another organism might be found in ninety per cent of the stomachs. To offset this possible error, both percentage occurrence and the percentage of each organism in relation to the total of all organisms eaten are considered.

Originally the individuals of each of the three species in the collection were divided into two-inch (5 cm.) length groups in order to show whatever change in food might be correlated with increase in size of the fish. The tabulations prepared therefrom are included herein only for the "river" forms and such findings as the data revealed for the "lake" specimens are indicated in the discussions. Separation has been made between lake- and river-caught specimens in order to compare the food in these different environments.

RESULTS

In tables I, II and III, the food of each size group is shown for each of the three species taken in the river.

River Rainbow

No appreciable change in the type of food was observed as size of fish increased (table I). Insects were distinctly dominant for every size group, Simuliidae being eaten in greatest numbers, although Trichoptera were found in a larger number of stomachs.

River Cutthroat

In contrast to the rainbow trout the cutthroat showed a definite variation in food with increase in size (table II). Insects dominated in the diet of individuals up to 15.0 cm. (6 in.) in length. From 15 to 20 cm. (6 to 8 in.) insects, particularly Trichoptera, Ephemeroptera and Chironomidae, are the most impor-

tant, but fish appear in one-third of the stomachs. Thereafter fish are the principal food, with insects becoming progressively less frequent.

Of the insect food, Chironomidae for the small cutthroat and Trichoptera and Simuliidae for the larger ones are the most important. Ephemeroptera and Plecoptera are less frequent.

Brown

The brown trout (table III) captured in this investigation do not represent as complete a series as the other two species. No brown trout were captured in

Table I. Analysis of food of rainbow trout, caught in the river, arranged according to size of fish and types of organisms eaten. A,—total number of organisms eaten; B,—per cent of total number eaten; C,—per cent of stomachs containing this organism.

			AQUATIC										TERRESTRIAL						
Length in cm.	N.		Trichoptera	Simuliidae	Chironomidae	Ephemeroptera	Plecoptera	Odonata	Hydracarina	Amphipoda	Gastropoda	Miscellanea	Coleoptera	Homoptera & Hemiptera	Formicoidea & Apoidea	Isoptera	Miscellanea	Fish	Fish eggs
0 - 5	12	A	6	2	193	15	2							4					
		B	2.7	0.9	86.5	6.7	0.9							1.8					
		C	33.3	8.3	91.6	91.6	16.6							16.6					
5 - 10	3	A	1	2	23	6	32												
		B	1.6	3.1	36.0	9.4	50.0												
		C	33.3	33.3	100.0	100.0	100.0												
10 - 15	48	A	379	500	11	61	11			2		10	4		10		3	16	
		B	37.1	48.9	1.0	5.9	1.0			0.2		1.0	0.3		1.0		0.2	1.5	
		C	85.5	31.4	18.5	27.0	12.5			2.5		9.0	8.0		6.0		6.0	4.0	
15 - 20	55	A	346	37	13	40	40	2	8	3	13		12	7	196	6	7	3	
		B	48.4	4.9	1.7	5.1	5.1	0.2	1.0	0.4	1.7		1.6	0.8	27.6	0.8	0.8	0.4	
		C	82.7	25.0	11.5	34.0	34.0	3.8	5.7	5.7	3.8		17.3	7.6	28.7	3.8	5.6	3.8	
20 - 25	30	A	223	5574	25	23	13	8		1	88		203	6	238	22	4	1	
		B	3.4	85.1	0.5	0.3	0.2	0.1			1.3		3.1		5.3	0.3			
		C	86.6	60.0	36.6	13.3	16.7	23.3		3.3	10.0		30.0	16.7	36.7	3.3	13.2	3.3	
25.5 - 30.5	32	A	727	2994	6	16	26	2			58	6	22		748		13	4	
		B	16.0	66.0	0.1	0.3	0.5				1.2	0.1	0.4		14.3		0.3		
		C	78.1	37.5	12.8	25.0	18.7	3.0			12.5	9.0	25.0		31.0		27.3	6.0	
30.5 - 35.5	18	A	401	3720	33	15	11	4			51	2	122		49		14		1
		B	9.0	84.0	0.7	0.2	0.3	0.1			1.1		2.7		1.0		0.3		
		C	72.1	55.5	33.0	22.0	37.7	16.5			16.0	11.0	27.7		44.0		55.0		5.5
35.5 - 40.5	8	A	209	1774	40	2	18				74	1				6		5	33
		B	11.4	81.9	1.8		0.8				3.4					0.3		0.2	1.5
		C	37.5	25.0	25.0	25.0	12.5				12.5	12.5				12.5		12.5	12.5
40.5 - 45.5	9	A	109	5208	8	20	15				1		4		12		2		
		B	1.9	96.7	0.1	0.3	0.2								1.1				
		C	100.0	66.6	44.4	33.3	22.2				11.1		22.2		11.1		22.2		
45.5 - 51.0	5	A	24	677			6				3		1		14				
		B	3.1	93.6			0.8				0.4		0.1		1.8				
		C	40.0	40.0			40.0				40.0		20.0		40.0				

the lake and the sizes between 10 and 25 cm. (4 and 10 in.) are incompletely and unsatisfactorily represented. For the fry and fingerlings up to 10 cm. (4 in.) the food was no different from that of the rainbow and cutthroat of this size. It consisted entirely of insects, mostly Chironomidae and Ephemeroptera. For the larger fish, 31 cm. (12 in.) and longer, both insects and fish took an important place in the food. Insects were dominant in specimens up to about 45.5 cm. (18 in.) and fish in larger individuals.

Lake Rainbow

Owing to the smaller number (32) of lake rainbow trout stomachs examined, no great significance can be attached to any differences shown in food with the

size of the trout. While Trichoptera were the most important food for the greatest number of size groups (five out of eight groups), terrestrial Coleoptera ranked first with two groups and snails with one. Only three of the eight groups ate fish. These were 20 to 25.5 cm. (8 to 10 in.), 25.5 to 30.5 cm. (10 to 12 in.) and 40.5 to 45.5 cm. (16 to 18 in.). Hence there seemed to be but little more tendency for the food of the lake rainbow trout to change with size than in the case of the river rainbow. Fish were somewhat more important as food for these trout than

TABLE II. Analysis of food of cutthroat trout, caught in the river, arranged according to size of fish and types of organisms eaten. A,—total number of organisms eaten; B,—per cent of total number eaten; C,—per cent of stomachs containing this organism.

Length in cm.	N.		AQUATIC												TERRESTRIAL					
			Trichoptera	Simulidae	Chironomidae	Ephemeroptera	Plecoptera	Tipulidae	Odonata	Hydracarina	Ostracoda	Amphipoda	Gastropoda	Miscellanea	Coleoptera	Homoptera and Hemiptera	Aphidae	Formicoidea and Apoidea	Miscellanea	Fish
0 - 5	12	A	4		144	28	3				6			1						
		B	2.1		77.3	15.1	1.6				3.3			0.5						
		C	33.3		75.0	83.3	25.0				8.3			8.3						
5 - 10	8	A	7		6	10	4	1							3		6	7	3	
		B	14.9		12.7	21.2	8.5	2.1							6.3		12.7	14.9	6.3	
		C	50.0		50.0	83.3	33.3	16.6							33.3		33.3	66.6	50.0	
10 - 15	23	A	33	25	394	82	21							6	3			1	8	108
		B	4.8	3.6	57.8	12.0	3.0							0.6	0.3			0.1	1.0	13.6
		C	30.4	21.7	39.1	47.8	30.4							12.9	4.3			4.3	21.4	43.4
15 - 20	27	A	56	10	24	11	3		3	3			18		4	1		9		63
		B	27.3	4.8	11.7	5.3	1.4		1.4	1.4			8.7		1.9	0.4		4.3		30.7
		C	51.9	7.4	25.9	29.6	7.4		7.4	3.7			3.7		14.8	3.7		7.4		33.3
20 - 25.5	14	A	35	66	40	7	1		2			1	2		1			21	2	31
		B	16.3	31.5	19.1	3.3	0.5		1.0			0.5	1.0		0.5			10.4	1.0	15.4
		C	64.1	14.2	14.2	14.2	7.1		14.2			7.1	7.1		7.1			14.2	14.2	92.8
25.5 - 30.5	17	A	56	590	14	2			1									1		32
		B	7.9	84.7	1.9	0.2			0.1									0.1		4.5
		C	35.2	41.1	29.3	11.6			5.8									5.8		94.1
30.5 - 35.5	9	A	12			1												13	1	10
		B	32.4			2.7												35.0	2.7	27.0
		C	53.5			11.1												11.1	11.1	44.4
35.5 - 40.5	7	A	2	43		31	4													32
		B	1.8	38.5		27.6	3.5													28.5
		C	28.4	28.4		14.2	28.4													82.7
40.5 - 45.5	2	A	7		2															9
		B	38.7		11.1															50.0
		C	100.0		50.0															100.0
45.5 - 51.0	1	A	1																	
		B	100.0																	
		C	100.0																	

for the river specimens, but no conclusive evidence is apparent that the larger trout eat relatively more fish than the smaller ones.

LAKE CUTTHROAT

Again the smaller number (32) of specimens makes analysis of the food of the lake cutthroat difficult. This small number probably accounts for the somewhat surprising implication that the lake cutthroat seemed to turn to a dominantly fish diet at a larger size than do those in the river. Not until a size of 41 cm. (16 in.) was reached did these trout eat fish in any number. The largest cutthroat appeared to subsist entirely on fish, chiefly kokanee. Otherwise their food differed little from that of the rainbow, with insects, mostly caddisflies and terrestrial Coleoptera, being the most important food up to 41 cm. (16 in. in length).

PREDATORY TENDENCIES OF COWICHAN TROUT

Throughout this investigation special attention has been paid to the fish-eating tendencies of the trout for the following reasons:

First, in evaluating the success of the brown trout plantings in the Cowichan river, probably the most important factor to consider is the effect of this species on the other fishes of the river system. Hardly anyone will deny that the destruction of the populations of native rainbow and cutthroat trout and the various species of salmon would be too great a price to pay for the presence of brown

TABLE III. Analysis of food of brown trout, caught in the river, arranged according to size of fish and types of organisms eaten. A,—total number of organisms eaten; B,—per cent of total number eaten; C,—per cent of stomachs containing this organism.

			AQUATIC									TERRESTRIAL			
Length in cm.	No.		Trichoptera	Simulidae	Chironomidae	Ephemeroptera	Plecoptera	Odonata	Cladocera	Gastropoda	Miscellanea	Coleoptera	Formicoidea and Apoidea	Miscellanea	Fish
0 - 5	20	A	2	3	116	17	1		1			9			
		B	1.3	2.0	77.8	11.4	0.6		0.6			6.0			
		C	10.0	15.0	80.0	50.0	5.0		5.0			5.0			
5 - 10	15	A			3	9									8
		B			15.0	45.0									40.0
		C			28.5	71.4									57.1
10 - 15	3	A	21		8	20									
		B													
		C	100.0		100.0	100.0									
15 - 20	2	A	7			1									15
		B	31.8			4.5									63.7
		C	33.3			33.3									100.0
25.5 - 30.5	2	A	44	1				3							
		B	91.6	2.0				6.0							
		C	100.0	50.0				50.0							
30.5 - 35.5	8	A	75	192	1	2				24	1		4		6
		B	24.9	62.8	0.3	0.6				7.8	0.3		1.3		1.7
		C	75.0	25.0	12.5	25.0				12.5	12.5		12.5		50.0
35.5 - 40.5	18	A	239	87	3	11	221			68	3	1	1		24
		B	36.7	11.7	0.4	1.6	34.1			10.4	0.4	0.1	0.1		3.7
		C	88.8	11.1	5.5	27.7	11.1			22.2	16.6	5.5	5.5		44.4
40.5 - 45.5	25	A	1	392		10	7	5		18		6	12	6	60
		B	0.9	62.9		1.6	1.9	0.7		2.8		1.0	1.9	1.0	9.6
		C	11.1	16.0		12.0	16.0	12.0		8.0		12.0	12.0	4.0	80.0
45.5 - 51.0	9	A	34			1	22	1							14
		B	46.5			1.3	30.5	1.3							19.4
		C	33.3			11.1	22.2	11.1							66.6
51.0 - 56.0	8	A	18							41	6	2	3		14
		B	21.1							48.3	6.6	2.3	3.3		16.5
		C	56.8							14.2	85.2	14.2	42.6		71.4

trout, regardless of how fine a game fish the latter might prove to be. One of the most obvious methods of determining this effect is to find what numbers of the native fishes are being directly destroyed by the brown trout as normal items of food.

Second, great interest has always been attached, both by anglers and fisheries workers, to the numbers of fish eaten by the various species of trout. Various opinions are advanced as to which species is the most piscivorous.

Third, it is desirable to determine the value, as food organisms, of the so-called "coarse" fishes. In the Cowichan river the stickleback (*Gasterosteus aculeatus* L.) and the sculpin (*Cottus asper* Rich.) are the species chiefly involved. The food of these fish has been shown to be very similar to that of the trouts, (Munro and Clemens 1937; Nurnberger 1930; Greely 1927; etc.). Hence they are food competitors of the trout and, since the food of any body of water is limited, it would at first seem desirable to remove these "coarse" fish. However, since they are eaten by the trout it is possible that their value as food more than offsets the harm they do as food competitors. A careful evaluation of their place in the trout diet is therefore necessary.

A fair comparison of the relative fish-eating proclivities of the three species can only be made when conditions are as nearly alike as possible. Habitat and size of the trout are the two factors that have the greatest effect on the number of fish eaten. Therefore, since no brown trout were captured in the lake, it seems advisable to limit discussion to the stomach contents of river trout of from 25.5

TABLE IV. Fish eaten by river trout, 25.4 cm.-50.8 cm. (10-20") long

	104 rainbow		37 cutthroat		67 brown	
	No. eaten	Total no. eaten (%)	No. eaten	Total no. eaten (%)	No. eaten	Total no eaten (%)
Salmon			10	11.6	6	7.7
Trout			2	2.3	1	1.2
Unknown salmonids	9	90	20	23.2	31	39.7
Sculpins			1	1.2	3	3.8
Stickleback			47	54.6	22	28.4
Unidentified	1	10	6	6.9	15	19.2
Total	10	100	86	99.8	78	100.0
Occurrence (%)	3.8		78.3		58.2	
No. eaten (av.)	0.09		2.32		1.16	
Salmonids (%)	90.0		37.4		48.6	
Coarse fish (%)			55.8		32.2	
Unidentified (%)	10.0		6.9		19.2	

cm. (10 in.), which is the lower size limit of the main group of brown trout, to 51.0 cm. (20 in.), the upper size limit of the rainbow trout.

Within this category, those individuals consuming fish were as follows: of 104 rainbow trout, 4 (3.8%); of 37 cutthroat, 29 (78.3%); and of 67 brown trout, 39 (58.4%). On this basis the results show that fish form an insignificant proportion of the food of the rainbow trout, are important as food for the brown trout, and exceedingly important for the cutthroat trout.

In table IV the general identification of the fish consumed by the trout studied is given, as closely as such separation could be made. It is seen that the brown trout ate a greater proportion of salmonids than the cutthroat. This differ-

ence is probably even more marked than is shown since more fish eaten by the brown trout are designated as "unknown", and undoubtedly a large proportion of these were salmonids. Hence, if we are to judge by the somewhat scanty data, brown trout are more inclined to eat salmonids than are cutthroat trout and eat proportionately fewer "coarse" fish, leaving these to act as food competitors of the salmonids. Cutthroat, on the contrary, eat a great many more sticklebacks than the brown trout. It is apparent that sculpins do not form an important item of food for any of the trout, sticklebacks are eaten extensively, and salmonids are heavily consumed.

Winter Feeding

The foregoing study embraces only material obtained during the summer (May to September). During the winter (October to February) of 1940 and 1941, collections of trout stomachs were continued by Mr. Ferris Neave and the data kindly furnished for inclusion herein (table V).

Rainbows were well represented but for cutthroat and brown trout the few specimens examined make the food study merely indicative of the probable seasonal trend. Salmon eggs, mainly from coho salmon spawnings, constitute

Table V. Winter food of 42 river trout. A=total number of organisms eaten; B=per cent of total number eaten; C=per cent of stomachs containing this organism.

Organism	35 rainbow (20–35.5 cm.)			4 cutthroat (26–40.5 cm.)			2 brown (41–5.55 cm.)		
	A	B	C	A	B	C	A	B	C
Trichoptera (larvae, pupae, adults)	47	7.4	34.3	2	3.0	20			
Simuliidae " " "	26	4.1	11.3	10	14.7	20			
Chironomidae " " "	10	1.6	22.7						
Plecoptera (nymphs, adults)	4	0.5	5.6						
Arachnida	3	0.4	5.6						
Gastropoda	11	1.7	5.6						
Salmonid eggs	529	84.0	65.6	44	65.3	20	3	75	50
Trout				12	17.0	60	1	25	50
Stickleback									

the principal food during this period, particularly for the rainbows, although insects were still important. The cutthroats still continued to consume young fish while the two brown trout stomachs contained only salmon eggs and young trout.

These data are of value as indicating quite clearly the change in the diet of the Cowichan trouts when the salmon spawnings occur and salmon eggs become available.

DISCUSSION

The present findings, which reveal the prime importance of insects and the negligible importance of fish in the diet even of the largest specimens of rainbow trout, are in general agreement with most investigations of rainbows in other regions, e.g. northern Utah (J. G. Needham 1933), Oregon (103 fish, Dimich and

Mote 1934), Vermont (49 fish, Lord 1934), Washington (95 fish, Doudoroff 1935), New York (80 fish, P. R. Needham 1938b).

The importance of Crustacea has been demonstrated in certain localities, e.g. Paul lake, B.C. (450 fish, Mottley and Mottley 1932; 161 fish, Rawson 1934), West Virginia (77 fish, Surber 1933), lake Arrowhead, California (28 fish, P. R. Needham 1938a), Michigan (451 fish, Metzelaar 1928). Crustacea make only a very slight contribution to the food of rainbows in the Cowichan river system.

Certain authorities have also observed the presence of fish in the diet to a considerably greater extent than is indicated by the present investigation. Juday (1907) found that while much insect food had been consumed by 106 rainbow trout from Colorado, fish remains were present in 42 stomachs. Metzelaar (1928) reported for the food of 451 rainbow trout in Michigan, that small trout subsisted almost entirely on insects, larger specimens on Crustacea and fish, while the largest turned entirely to fish and crayfish. Fish were noted in 33 of the stomachs. Mottley and Mottley (1932) found that 4 large Kamloops trout caught in Little river, B.C., had a total of 190 spring salmon (*Oncorhynchus tschawytscha*) fry in their stomachs, besides insects. Of 200 Kamloops from Kootenay lake, B.C., 18 had fed upon kokanee (*O. nerka kennerlyi*) and one on a young trout. Clemens (1939) stated that 28 Kamloops trout from Okanagan lake, B.C., had eaten mostly terrestrial insects and fish (kokanee).

It is of interest to note that four investigators (Metzelaar 1928 and 1929, Surber 1933, Lord 1934, and P. R. Needham 1938b) have drawn attention to the possible importance of algae in the diet, Metzelaar (1929) reporting it to amount to 15% of the food. In the present investigation algae were not recognized as of any significance.

Concerning cutthroat trout, the food studies are much fewer. Juday (1907) reports that the food of 72 cutthroats in Colorado consisted mainly of insects and crustaceans, though four had eaten fish. Muttkowski (1925, 1929) showed that the food of Yellowstone Park cutthroat was principally Plecoptera, with Ephemeroptera and Trichoptera next. For small stream cutthroat of the Rocky Mountain region of Montana and Wyoming, Hazzard and Madson (1933) found that insects were the chief food but that lake cutthroat ate more fish than did stream-dwelling individuals. Fish occurred in 38.8% of the stomachs, and an increase in the proportion of fish eaten was correlated with an increase in the size of the trout. Dimich and Mote (1934) in Oregon showed that Ephemeroptera and Trichoptera constituted the main food of 326 cutthroat trout but these trout had also eaten 126 fish.

These reports suggest that insects are the principal food of cutthroat trout generally, with fish somewhat more important than in the diet of the rainbow trout. The present investigations indicate that in the Cowichan, fish constitute as much of the food as insects and even more for the larger individuals.

Reports on brown trout are scanty, although several investigations have been conducted in the last few years. In North America, insects have been found to constitute the greater part of the food in the Genesee river system (10 fish, Greely 1927), Oneida county, N.Y. (16 fish, Clemens 1928), central New York state (46 fish, P. R. Needham, 1938b). In Michigan, Metzelaar (1929) from an

examination of 191 stomachs concluded that insects were the main food of trout up to nine inches long. Larger trout turned increasingly to "crab" and fish diet.

In the British Isles, Clarke (1924) showed that Diptera, mayflies, caddisflies and beetles were the main food of 749 brown trout from the upper Derwent. A few fish (cottids and trout fry) were eaten. Southern (1932) on the basis of 140 trout from two Irish localities distinguishes between the feeding habits of fish in limestone waters and in acid waters, those from the former type of habitat eating insects, crustaceans and small fish (mostly perch fry), whereas the trout from acid waters had eaten insects and crustaceans but no fish. Frost (1939) showed that 228 trout from the Straffan (an acid stream) ate chiefly Diptera and Trichoptera larvae and Ephemerid nymphs. Also 349 trout from the Ballysmutten (an alkaline stream) ate mostly plecopterous nymphs, Diptera and Trichoptera. Some fish (minnows and gudgeon) were eaten by the larger fish. Seasonal changes in diet were shown by 339 brown trout from Windermere (Allan 1938), the main food being molluscs and crustaceans from October to February, immature insects from March to July and surface insects from May to September.

According to these data, insects proved to be the main food of the brown trout in other areas. For the Cowichan, however, while insects may predominate slightly, fish are of definite importance. Trichoptera are the most important insect food, while salmonids are the principal fish consumed.

FOOD SELECTION BY TROUT

Since in this study three different species of trout have been involved, the opportunity is presented of giving consideration to the probable tendency among trout to discriminate among possible food organisms and practise some degree of selection. According to Neill (1938), "the trout feeds on the whole range of animals present in whatever type of habitat it finds itself, to an extent dependent on their degree of accessibility and the extent of their representation in the fauna". On the other hand, Allan (1938) states that "percentages which the most important food animals make up of the food are probably very much greater than are their percentages in the fauna at the same time, i.e., the fish are performing definite selection in their food".

The present results tend to suggest a definite discrimination on the part of the rainbow, cutthroat and brown trouts of the Cowichan river. Obviously the food is restricted to the organisms available in the environment, but within this environment it is found that the diets of the three species differ decidedly. Selection is thus indicated.

SUMMARY

During the summers (May to September) of 1937 and 1938, a collection of 281 rainbow trout (*Salmo gairdneri* Rich.), 171 cutthroat (*S. clarkii* Gibb.) and 140 brown trout (*S. trutta* L.) stomachs was made. During the winters (October to February) of 1940 and 1941, 35 rainbow, 4 cutthroat and 2 brown trout were obtained. The stomach contents were examined and the particular diets of the fish determined, not only according to species, but also for definite size groups. A separation has also been made between river and lake-dwelling individuals.

For rainbow trout, insects are the most important summer food, Trichoptera and Simuliidae predominating. For lake specimens there is evidence that fish may be more important than for river trout, but insects still form the bulk of the food. During the winter salmon eggs are of definite importance. No change in diet occurs as the fish increase in size.

For cutthroat trout, fish are equally as important as insects. Trichoptera are the principal insect food and sticklebacks are the major fish consumed. Salmon eggs are an important food constituent in winter. Up to a length of approximately 15.0 or 17.5 cm. (6 to 7 in.) insects form the main diet but for larger sizes fish become the dominant food. Lake cutthroat appear to have a larger percentage of fish in the diet than river-dwelling individuals.

For brown trout, up to 10 cm. (4 in.) in length, insects predominate. Fish form an important part of the food of large individuals. Trichoptera are the most frequent insect food, while salmonids are the dominant fish food. Little change in food occurs with increase in size from 30.5 to 50.8 cm. (12 to 20 in.) and in view of the inadequate representation of intermediate size groups in the collection, it is not possible to indicate when the change in diet, notably the consumption of fish, commences.

The data for the three species in the Cowichan river suggest that discrimination among possible food organisms may occur and that definite selection may therefore take place.

ACKNOWLEDGEMENTS

The writer is indebted to many people for assistance in this investigation. To Dr. W. A. Clemens, former Director of the Pacific Biological Station, Dr. R. E. Foerster, present Director, Mr. Ferris Neave, in charge of the Cowichan river research, Dr. G. C. Carl, formerly associated with the Cowichan river investigation, Dr. C. McLean Fraser, former head of the Department of Zoology, University of British Columbia, and Professor G. J. Spencer, Assistant Professor, sincere thanks are expressed for their very generous aid and helpful suggestions.

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New Records of Pycnogonida from the Canadian Atlantic Coast

By Louis Giltay

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(Owing to the author's death, these records have been excerpted from his manuscript and arranged by Alfreda Berkeley Needler)

(Received for publication February 13, 1942)

ABSTRACT

Nymphon glaciale, N. serratum, Pallenopsis longirostris and *Ammothea scabra* are recorded for the first time from the Canadian Atlantic coast.

The Pycnogonida of the Atlantic coast of Canada have never been extensively collected. In material sent me by Dr. A. G. Huntsman were the following species, which are here recorded for the first time as in the Canadian fauna.

1. Nymphon glaciale Liljeborg. (Liljeborg 1851, Sars 1891, Schimkewitsch (*N. grossipes* var. *glaciale*) 1930).

Canadian Records. Three specimens, gulf of St. Lawrence near Magdalen is., sandy bottom, 40 to 45 m., shrimp trawl.

Distribution. A circumpolar arctic species known from the sea of Ochotsk, Bering sea, sea of Karsk, cape Konin, the White sea, coast of Murmansk, coast of Norway, Spitzbergen, Franz Joseph island and Greenland.

2. Nymphon serratum G. O. Sars. (Sars 1879 and 1891, Schimkewitsch 1930, Stephensen 1933).

Canadian Record. One specimen, Hudson bay (mouth of James bay), gravel bottom, 75 m., trawl.

Distribution. An arctic species previously found in the north Atlantic from the coast of Siberia to Greenland.

3. Pallenopsis longirostris Wilson. (Wilson 1881).

Canadian Record. Two specimens (1 ♂, 1 ♀) Cabot strait, muddy bottom, 378 m., young fish trawl.

Distribution. Only one previous record of two specimens taken off the New Jersey coast at 915 m.

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4. Ammothea scabra (Wilson). (Wilson 1880).

Canadian Record. One specimen, ♀, gulf of St. Lawrence near Magdalen is., sandy bottom, 40-45 m., shrimp trawl.

Distribution. Two previous records, 1 ♂ from cape Ann, and 1 ♀ from St. George's bank.

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Effect of Temperature on Activity of *Salvelinus fontinalis*

By Paul F. Elson
Fisheries Research Board of Canada

(*Received for publication February 27, 1942*)

ABSTRACT

The distances moved in response to varied stimuli are measured, at constant temperature, by a new method. A sigmoid curve shows the relation between strength of stimulus and distance moved. For any strength of stimulus maximal amount of movement occurs at about 10° C. Laboratory experiments and field observations reveal response temperatures (more active behaviour of the fish) at 15 and 20°, which may somewhat increase the distance moved at and above these temperatures.

It is a general rule that a rise in the metabolic rate of an organism is concomitant with a rise in temperature. Therefore, since poikilothermal animals have a body temperature approximating that of their environment it might be expected that the activity of such animals would increase, within the normal temperature limits of the organism, as the temperature increased. However, while investigating the effect of water currents on the behaviour of speckled trout (*Salvelinus fontinalis* Mitchill) it was found that the fish exhibited noticeably less activity at a temperature of 20° C., which is well below the lethal temperature, than at a somewhat lower temperature (Elson 1939). Because temperature has a pronounced effect on the activity of trout, a knowledge of the relation between temperature and activity is a prerequisite for a complete understanding of the effects of current, and many other factors, on the fish in its natural environment.

METHOD

It was found by experimentation that when a trout was placed in a long, narrow trough and stimulated by passing an electric current through the water in the trough, the fish moved a distance which bore a fairly constant relation to the strength of the stimulus administered and to the temperature of the water in the trough.

Apparatus

The apparatus (figure 1) consists of a wooden trough (T) 48 inches (121.92 cm.) long, 2 inches (5.08 cm.) wide and 3 inches (7.62 cm.) deep, the inside painted black and the bottom marked off in 1- and 6- inch (2.54 and 15.24 cm.)

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intervals with white lines. A fairly constant temperature was maintained in the trough by means of a slight current of water from a constant-temperature tank. While this introduced a factor of current it was of small value and was kept constant. The actual rate of flow through the trough was $2\frac{1}{2}$ inches (6.35 cm.) per minute, resulting in an exchange of 24.4 cubic inches (400 c.c.) of water per minute. Air bubbles were not allowed to enter the trough.

The trough was illuminated by two 25-watt bulbs (L) suspended in a white box (A) which was as long as the trough. A piece of ground glass (P) was

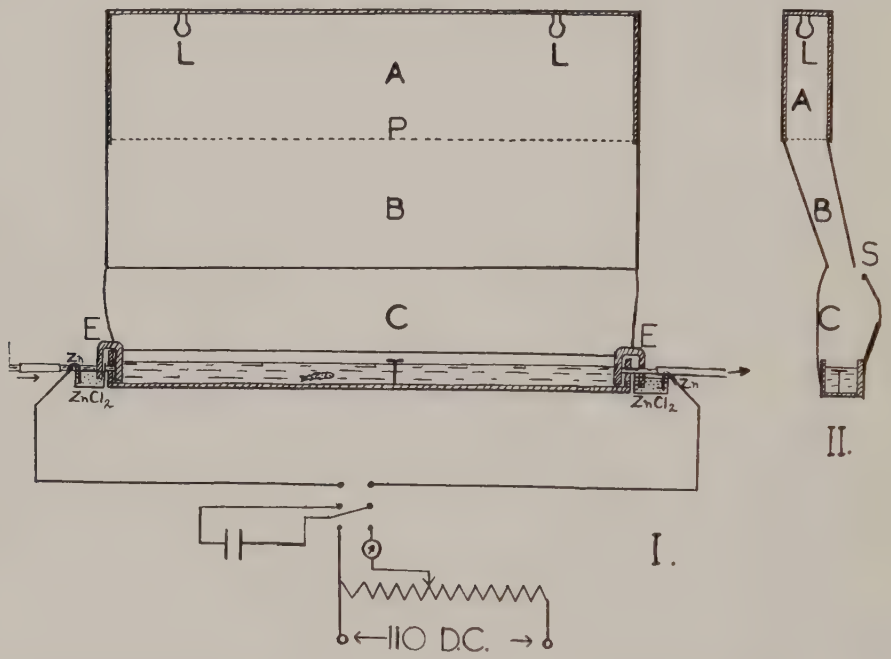

FIGURE 1. Apparatus used in studying the effect of temperature on the amount of movement resulting from an electrical stimulus. I.—side view of apparatus; II.—end view. A,—box for the lights; B,—metal sheets; C,—cotton sheets; E,—electrodes; L,—electric lights; P,—ground glass plate; S,—observation aperture; T,—trough with water and fish.

placed across the open bottom of this box to give diffuse and fairly uniform light to the whole length of the trough. Two sheets of galvanized metal (B), painted black, limited the amount of light reaching the trough. The lighting box was suspended slightly to one side of the trough. Between the metal plates and the trough was fastened a double thickness of black cotton (C) to exclude all outside light. An aperture (S) about 24 inches (60.96 cm.) long and 2 inches (5.08 cm.) wide was left between the lower ends of the plates and the upper edge of the cloth. Observation of the fish was made through this aperture with very little disturbance of the animal. Except for the bulbs above the trough and a well-shaded 25-watt bulb, used for making notes, the experiment was carried out in a darkened room.

An electrical stimulus was obtained by passing the discharge of a four microfarad paper condenser through the water in the trough. The stimulus was conducted from non-polarizable zinc - zinc chloride electrodes (E) to the trough through wooden (oak) bridges which had been saturated in one per cent sodium chloride and blackened by soaking in a hot dilute suspension of charcoal in agar. The ends of the bridges placed in the trough were wide enough to form false ends to the trough, with sufficient clearance to allow a slow flow of water past, and reduced the length of the trough available for movement of the fish to 44 inches (111.76 cm.). The strength of the stimulus was varied by charging the condenser to different voltages from a potentiometer (total resistance 355 ohms) connected to the 110 volt d.c. supply of the laboratory. A double-pole-double-throw switch was arranged so that the condenser could be charged from the source and discharged through the non-polarizable electrodes and the water in the trough. The strength of stimulus refers to the potential to which the condenser was charged.

Procedure

The fish used in the experiment was held in the trough, with constant light, for several days previous to commencing observations. Subsequently the fish exhibited much movement only when stimulated, as by a discharge through the electrodes.

When water temperatures were changed by 5° C. or more the fish became more active and remained so for not over two hours. Measurements of distance moved were not made during this interval.

The distance moved along the trough by the trout when it was subjected to the electrical stimulus was measured by means of the graduations on the bottom of the trough. Stimuli were administered at each of 15 voltages ranging from 2 to 110 volts. After a 2-minute rest for the fish, similar readings were made with decreasing voltages. After a 5-minute rest, the whole procedure was repeated. In this way ten measurements of distance moved were made for each strength of stimulus.

For the relation of distance moved to temperature, a series of 50 consecutive measurements, using a stimulus of 82 volts (just sufficient to give nearly maximal movement) was made at each of 6 temperatures, ranging from 2.5 to 25.1° C.

RESULTS

The results presented are for a 12 cm. trout, the only individual used throughout the entire range of temperatures. Results from other fish, over smaller temperature ranges, were similar.

When the fish was subjected to the stronger stimuli, and particularly at temperatures of 15° C. and higher, there was frequently a second more prolonged period of activity following the initial burst. Only the first movement was considered as the immediate outcome of the stimulus for the purposes of this experiment. At these higher temperatures the animal was restless, often moving without any apparent external stimulus.

Effect of Stimulus Strength on Amount of Movement

There was considerable variability in the amount of movement for a given stimulus. For example, at 9.5° C. the 10 movements resulting from a stimulus of 34 volts ranged from no response at all to 43 inches, average value 6.3 inches, at 92 volts from 28 to 54 inches, average 45.3, and at 110 volts from 14 to 57 inches, average 43.7. Nevertheless, the averages (table I) for each of the 10 readings for a given strength of stimulus show a definite order in the relation between strength of stimulus and amount of response as measured by distance moved.

Table I. Average distance (in inches) moved by a 12 cm. speckled trout at various temperatures in response to electrical stimuli of different strengths. (1 inch = 2.54 cm.)

Temp. (° C.)	Strength of stimuli in volts														
	2	8	14	22	27	34	41	49	56	64	73	82	92	101	110
1.9							1.4	4.6	3.7	7.8	10.0	21.3	22.5	33.7	33.6
5.3		0.1	0.1	0.0	3.3	1.7	7.0	6.7	8.7	21.2	33.3	38.4	34.5	42.5	41.4
9.5		0.1	0.7	0.7	0.7	6.3	15.5	13.2	31.1	34.6	39.5	41.9	45.3	35.1	43.7
14.6		0.2	0.2	0.3	0.1	0.4	3.0	10.4	9.5	18.6	21.5	38.5	39.2	39.2	42.0
20.0			0.2	0.5	0.5	0.7	1.5	5.1	7.4	6.3	17.7	27.7	29.0	32.8	31.1
24.9			0.4	0.5	1.0	0.7	1.7	3.1	5.5	9.4	14.5	21.3	22.7	28.7	30.9

The curves obtained from these data (figure 2) are sigmoid and indicate a rapid rise in the amount of response as stimulus strength is increased from between 30 and 40 volts to between 80 and 100 volts. In each case this rapid rise in the amount of movement occupies a range of about 50 volts and an approximately maximal movement occurs with a stimulus of about 100 volts.

Effect of Temperature on Amount of Movement

The relation between distance moved and temperature (figure 2) is essentially the same at all strengths of stimulus. For any given strength of stimulus the distance moved is greater when the water temperature is in the region of 10° than when the temperature is either above or below this point (figure 3, dotted lines).

Also in the experiment involving 50 consecutive measurements of distance moved (table II) the maximal movement in respect to temperature occurred at 10° (figure 3, solid line). The curve drops at about the same rate going up from 10 to 15° as going down from 10°. From 15 to 25° it drops less rapidly.

Upper and Lower Temperature Limits for Activity

The trout used in these experiments had been held in a tank in which the water temperature was between 5 and 10° for upwards of 3 months. Death of these trout occurred within a few minutes between 26.5 and 28.0°, which limit for activity is in close agreement with the lethal temperature reported by Embody (1921). At 0.0° these trout oriented against the direction of rotation of a circular dish (method of Gray, 1937) but did not move through the water to maintain relation with the "bottom" pattern. At −0.5° all orientation failed.

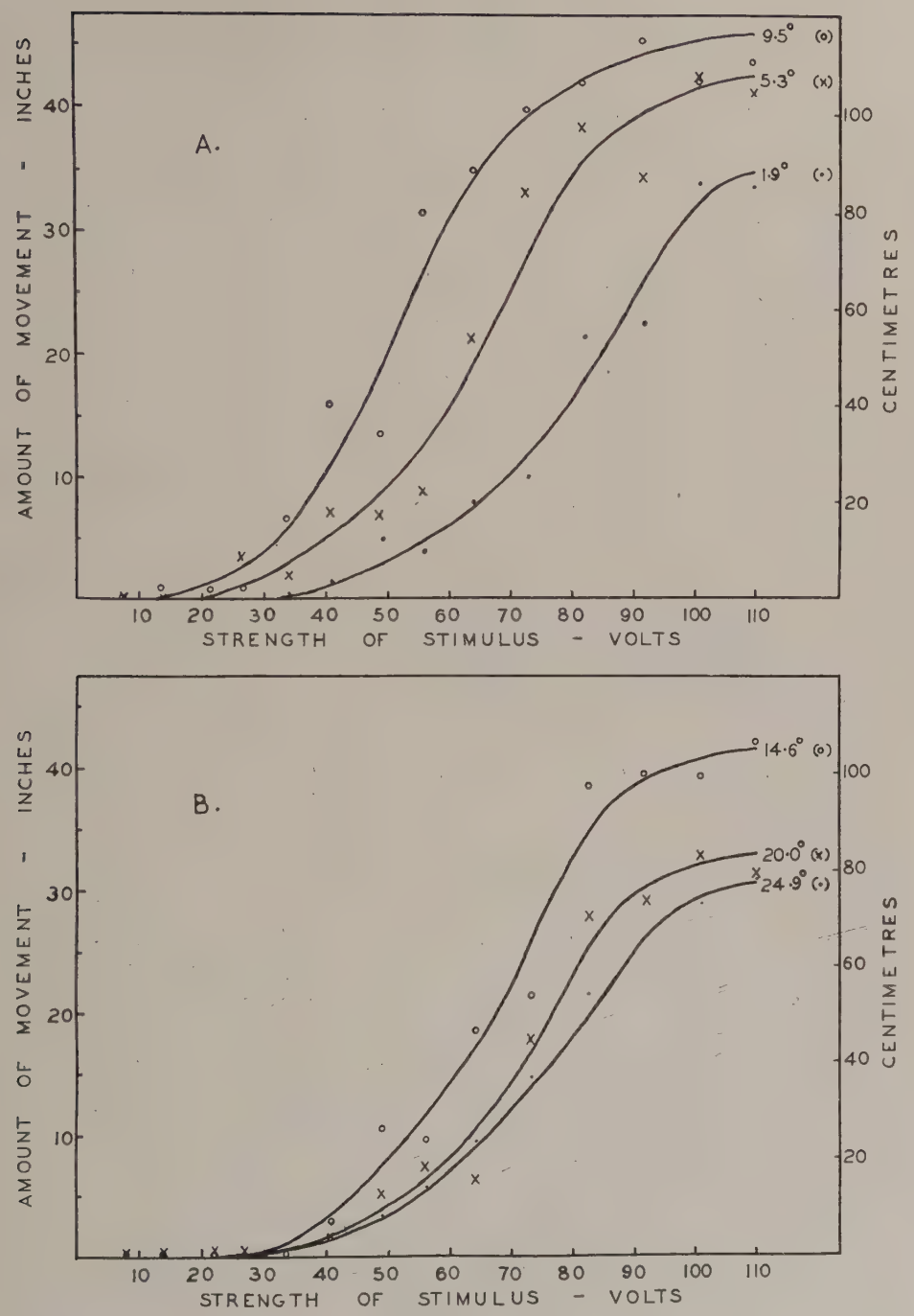

FIGURE 2. Relation between strength of stimulus and resulting amount of movement at various temperatures. A,—at 1.9, 5.3 and 9.5° C.: B,—at 14.6, 20.0 and 24.9° (data from table I).

TABLE II. Distance moved, at various temperatures, by a 12 cm. speckled trout subjected to an electrical stimulus of 82 volts. (1 inch = 2.54 cm.)

Temperature (° C.)	Distance moved (mean value of 50 observations) (inches)	Standard deviation (inches)	Coefficient of variability (%)
2.5	19	13	68
4.9	31	14	45
10.0	42	11	26
14.7	32	13	41
20.1	26	13	50
25.1	23	16	70

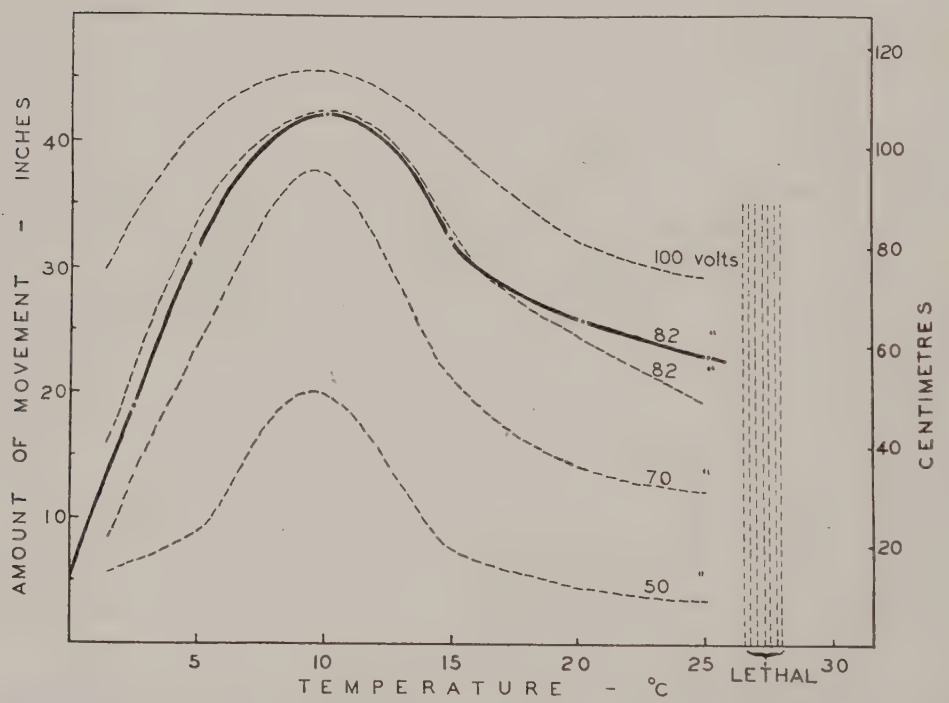

FIGURE 3. Relation between temperature and distance moved in response to an electrical stimulus. Broken lines are based on values from the curves in figure 2, for stimuli of 50, 70, 82 and 100 volts; solid line is based on the data in table II.

RESPONSE TEMPERATURES

Rubin (1935) demonstrated that certain fishes "became markedly active at specific 'response temperatures'." In an experiment similar to his a speckled trout was placed in a vessel containing water at 10°. After the fish had become quiet and remained so for 5 minutes, hot water (about 80°) was allowed to rise around the vessel to the level of the water inside. This warmed the water, in which the trout was, at a rate of 2 to 3° per minute.

Between 10 and 15° the fish was motionless. At 15° it commenced slow wandering movements; at a temperature between 20 and 21° it became violently active, swimming about and leaping. This activity was maintained as the temperature rose above 21°. At 25 to 26° the fish became distressed and was at once transferred to water at a lower temperature (18 to 20°). After recovery of the fish the experiment was repeated with similar results. As long as the temperature did not rise above 25° the fish showed no ill effects. If, when the temperature reached this point, the water was allowed to cool, the fish remained active until a temperature between 21 and 20° was reached, when the animal became almost motionless and remained so as the temperature dropped below this point. The experiment was repeated several times on each of 3 different fish. Each time there was a slight response at 15° and a very vigorous response at 21°.

This indicates that speckled trout have a "response temperature" at about 21°, and possibly also at 15°, at which they become unusually active as a result of temperature stimuli. Rubin showed by cutting the main nerve supply of the lateral line in his fish that these stimuli are received primarily through the lateral line system and are thus sensory stimuli. We have confirmed this for trout.

DISCUSSION

The activity of a fish subjected to a given stimulus under different conditions of water temperature will be dependent on two factors, first, the irritability of the animal, which is a result of the effect, if any, of temperature on the receptor organs, and second, the amount of movement resulting from the stimulus received, which is a result of the effect of temperature on the total number of receptor-effector units involved. This study deals primarily with the second factor.

The significance of the relation, which is described by a sigmoid curve, between strength of stimulus and magnitude of response is dependent on the precise mechanism by which the electric shock, in these experiments, arouses activity. There can be little question that the stimulus is effective through the surface sense receptors of the body and stimulation of these receptors results in the movement observed. In general the irritability of a given type of receptor is so distributed that the total number of receptors responding at each value of the stimulus increases in a manner illustrated by a sigmoid curve as the stimulus strength increases (Hoagland 1933). The fact that, in these experiments, the amount of response is similarly related to strength of stimulus, suggests a relation between irritability of the receptors involved and the responses of the organism. It is possible that not a single type of receptor but several types were involved in the experiments. Their respective irritabilities may still, however, be distributed according to the manner illustrated by a sigmoid curve. If such a curve does represent the distribution of the irritabilities of a number of separate sense organs, then, because of the apparent relation between irritabilities and responses, it is also implied that each sense organ has a certain fixed amount of response associated with it.

It is a common observation that the rates of many functions increase with increasing temperature, as for example, muscular contraction and glandular secretion. The irritability of a receptor increases with temperature, that is the stimulus necessary to produce a certain response decreases, with increasing temperature, up to the lethal point. This experiment shows that the amount of movement of a trout resulting from a given stimulus increases with increasing temperature up to about 10° C., which is below the midpoint between upper and lower temperature limits of the animal, and above this point decreases in amount with further increasing temperature. This increase and decrease in amount of movement with rising temperature can be explained on the basis of stimulation of some receptors resulting in excitatory responses, and stimulation of others causing inhibitory responses (the two types of responses having different temperature relations) rather than on some special, unknown property of receptors. Above 10° the inhibitory responses increase at a more rapid rate than the excitatory responses, with a consequent decrease in the activity of the organism as a whole.

The reactions of the trout indicate that approximately all the receptor-effector units were involved when a stimulus of 100 volts was employed across the electrodes, regardless of temperature.

Optimal temperatures for capture of fish have been reported by Beaugé (1929) for codfish, who gives an optimum of 4° in a range from 1.5 to 10°; and by McKenzie (1932) for haddock, who states that the best catches are made between 7.3 and 10.0° in a range from 2.2 to 11.9°. The amount of movement probably affects capture of these fish and the optimal temperatures for capture may well be related to maximal movement. This would correspond with the described behaviour of the speckled trout, it being understood that optimal temperatures may vary with the species and even with acclimation of individuals.

M'Gonigle (1931) studied the effect of temperature on the rate of respiratory movements of trout, a single function of the organism, not the organism as a whole. Above 10° the rate of respiratory movements increases more slowly than below this point, which is apparently a "critical temperature" in the sense of Crozier (1925-1926). However, while the rate of respiratory movements continues to increase to a temperature of 22°, the amount of movement of the fish resulting from a stimulus decreases above this "critical" value of 10°. A decrease in the rate of respiratory movements above 22° may be associated with the response temperature observed at about 21°.

While studying the behaviour of speckled trout in lake Ainslie, Nova Scotia, in 1938, under the auspices of the Fisheries Research Board of Canada, it was found that, other things being equal, the trout moved from the lake into a brook in greater numbers when the lake temperature was above 21° (figure 4), the brook being usually 4 to 6° cooler than the lake. Presumably this indicated greater activity on the part of such trout. An increase in such "spontaneous" activity must be a result either of more distance moved per stimulus or of more responses to more stimuli. Since it has been shown above that maximal movement per stimulus occurs at 10°, then the latter explanation must apply to this observation of increased activity at 21°. That is, at this temperature, tempera-

ture *per se* becomes a stimulus to movement, and this appears to be correlated with the increased movement of the lake Ainslie trout into the brook.

It seems probable also that it is reaction to the response temperature at 21°, and possibly to a similar effect at 15°, which causes above these temperatures more movement after a given stimulus than might otherwise occur (figure 3).

SUMMARY

Speckled trout in a long, narrow trough, when subjected to an electrical stimulus, move along the trough a distance which is fairly constant for an individual fish and for fixed strength of stimulus and constant temperature. Observations on the amount of this movement have been made at various strengths of stimulus and at various temperatures.

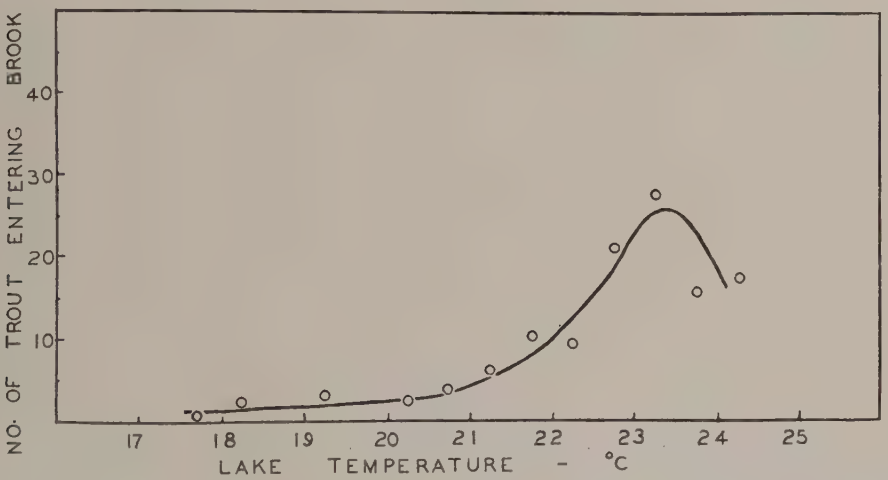

FIGURE 4. Relation between lake temperature and average number of speckled trout entering Trout brook from lake Ainslie, N.S., July to September, 1938.

The relation of amount of movement to strength of stimulus is expressed by a sigmoid curve.

The amount of movement resulting from any given stimulus at different temperatures is greatest at about 10° C., which is far below lethal temperatures.

At temperatures below 10° the amount of movement is progressively less, but trout can move about at 0° for several hours, at least.

At temperatures between 10 and 15° the amount of movement becomes progressively less about as rapidly as on the other side of the point of maximal movement.

Between 15 and 25° the amount of movement also becomes progressively less, but not so rapidly as at the corresponding place on the lower side of the point of maximal movement. This appears to be associated with a reaction to specific response temperatures, for speckled trout, at 21° and possibly also at 15°.

Field observations suggest a correlation between the numbers of trout migrating from a lake into a brook and the existence of such a response temperature of 21° in the lake.

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Sea Life of the Brook Trout (*Salvelinus fontinalis*)

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ABSTRACT

In the sea, brook trout travel in schools along the sea shore and feed largely upon small fishes of various species and upon Crustacea.

Previously (White 1940, 1941) we dealt for this species with the life history in fresh water and the migrations to and from the sea. The average time spent in the sea was found to be 64½ days or approximately two months. Some wandered eight miles (13 km.) or more from their home stream and ascended other streams. The vastness of their possible habitat in the sea and the relative paucity of their numbers increase the difficulty of obtaining information concerning their behaviour there, of which very little is known. On the other hand, the same factors give added significance to observations in the sea on small numbers of trout. Until the summer of 1941 we had neither seen nor taken them in the sea except at the head of the estuary soon after their descent of the river.

Outward Migration

The trout descend the river during April and May, when they are taken in the inner estuary in considerable numbers by anglers. As there is never any concentration of trout there, they evidently pass rather quickly out to the sea. But while in the inner estuary they start feeding actively, taking elvers, isopods and amphipods (White 1940). Four large trout caught at the head of estuary May 5, 1941, were gorged with sand worms (*Nereis* sp.). At this time the trout are in a transitional condition as to coloration between that of the parr and that of the full sea stage.

Behaviour in the Sea

We have been informed by local commercial fishermen that at times trout are seen in schools in water five to ten feet (1.5 to 3 m.) deep around the inner islands and also around some of the small private wharves along the mainland shore, where they congregate when lobster pots are brought in and cleaned or when fish are being dressed. Sometimes they are caught by angling from the wharves or in short lengths of gill net set near the shore. At some places, e.g. at the mouth of the Moser river estuary in June and July of 1941, trout are regularly taken by trolling along the shore.

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On May 18, we first observed the trout in sea water about 8 feet (2.4 m.) deep off a small wharf in a sheltered bay some four miles (6½ km.) west of Moser river, and caught two of them by using live mummichogs (*Fundulus heteroclitus*) for bait. The bottom around this wharf is a dark mud.

On June 20, 21 and 22, which is about the middle of their short stay in the sea, we found trout near the shore about five miles (8 km.) east of Moser river. That shore is typical of most of the shore of the district and is of bed rock or boulders and has a good growth of rockweed (*Fucus* sp.) covering most of the intertidal zone. The shore is wave swept and the water clear. The fauna and flora (lobsters, crabs, flounders, sculpins, starfishes and kelp) are those of the sea rather than those of the estuary or brackish water. At this place small loose schools of trout (as many as 20 and probably more in a school) were seen swimming rapidly but erratically against the set of the tide. So rapidly did the schools move that there was little opportunity to capture many by angling and on the average less than one fish per school was taken. The restless darting movements, probably associated with their very active feeding, were unlike those of any trout that we have ever seen in a freshwater habitat. There was a definite tendency for the fish to be in separate schools according to size, with fewer of the larger fish. Fine tackle (small hooks and fine leaders) seemed to be necessary for their capture in comparison with what is effective in fresh water.

The trout taken from the clear sea water of this rocky shore were lighter in colour than those we have seen elsewhere. The backs were a light blue-green with the dorsal fin greyish-green and mottled with grey. The sides were very silvery and the belly pearl-white. The pectoral, ventral and anal fins were almost pure white but the caudal fin was dark grey to nearly black. Their coloration blended so perfectly with the clear sea water that their movements were not easily followed, and even when being brought to the net it was sometimes difficult to see the outlines of the fish.

Table I. Food of trout in the sea near Moser river

Food organism	Frequency	Number	Volume (%)
Rocky shore (12 specimens)			
Urophycis chuss	10	54	73.6
Anguilla bostoniensis	2	3	0.8
Hemitripterus americanus	2	4	9.1
unidentified Cottidae	2	2	0.3
Fundulus heteroclitus	2	3	6.6
Pholis gunnellus	3	7	8.3
unidentified fish	3	5	1.3
Mud bottom (3 specimens)			
Crago septemspinosus	2	41	49
Mysis stenolepis	2	3	1
Gammarus sp.	1	1	5
Anguilla bostoniensis	1	2	30
Pholis gunnellus	1	1	15

Food in the Sea

Some years ago we took trout in the sea around Prince Edward Island and noted in their stomachs smelts, silversides, shrimps, amphipods, isopods and terrestrial beetles. Detailed analyses have been made of the stomach contents of the trout taken from the sea near Moser river (table I). Along the rocky shore the young of the common hake (*Urophycis chuss*), averaging about 65 mm. in length, formed the greater part of the food. Two of the larger trout contained 13 each, and several of the trout on capture disgorged young hake which could not be included in our figures. All the fishes taken by the trout were small (25-65 mm. long). The trout in the quieter bays having muddy bottom had fed largely upon Crustacea, especially the common sand shrimp (*Crago septemspinosus*) which is abundant in such locations.

Return to the River

Apparently they cease to feed actively on their return as fat fish to the river's mouth, and they are not fished until after they enter the river. Mr. D. G. Wilder reports for 87 trout caught in the river within a few rods (1 rod equals 5 m.) of the head of tide during their return migration, that the stomach of one was full and those of the two others half full of partly digested fish flesh. Twenty-nine had only traces of food in them and 55 were completely empty.

Conclusions

The sea habitat most frequented by these trout is seen as being along the rocky, wave-washed shores of the inner islands and mainland of this region.

In this habitat their food is practically confined to small fishes found in or near the beds of rockweeds.

In about two months they attain their maximum weight for the year, since on return to fresh water they lose weight.

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Vertebral Number of Young Herring in Southern British Columbia

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ABSTRACT

Statistical treatment of vertebral counts of young herring taken along the southeast coast of Vancouver island demonstrates significant differences between localities in the same year and in the same locality in different years. These differences are probably largely due to the effects of environment. The larger fish in a sample tend to have higher vertebral counts. This increase is somewhat greater in the caudal than in the abdominal region, and is accompanied by a disproportionate increase in mean number of tail vertebrae. Between samples, variation in total count is reflected mainly in the abdominal vertebrae, and significant differences in mean tail count may occur where no difference in total count exists.

INTRODUCTION

By comparing vertebral counts and other characters of adult herring (*Clupea pallasii*) from various localities along the coast of British Columbia, Tester (1937) has shown that differences exist in average vertebral number which are statistically significant, and therefore concludes that free intermingling does not take place. These general conclusions have been confirmed by the results of an extensive tagging programme. In general, the similarity of samples from particular localities was deduced by means of statistical procedures which demonstrated lack of heterogeneity in the vertebral data. These data suggested that only one population was being sampled in each area described, with the possible exception of Saltspring island, which is the main fishing ground along the southeast coast of Vancouver island.

Considering the origin of each "local population" it must be pointed out that probably no year class is the result of a single spawning. The eggs of the Pacific herring are generally deposited on vegetation in the intertidal zone (Hart and Tester 1934), and spawnings in one locality may be separated both in time and space. Since vertebral number is known to be affected by temperature (Tester 1938), it is possible that the products of separate spawnings, even from the same beach, may be shown to differ if compared on the basis of this character. Thus, in a fishery such as that exploited at Saltspring island, the mature fish of a single year class may have been recruited from the progeny of many different spawnings, each of which has undergone its early development under a different set of environmental conditions.

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A preliminary report on vertebral counts of young from various nursery grounds has already appeared (Hart and McHugh 1939).

MATERIAL AND METHODS

Samples of young herring in their first summer of life were collected in bays and channels along the southeast coast of Vancouver island. Schools located near shore were sampled by means of a small-meshed drag-seine; farther offshore, dynamite was used; and occasional samples were taken with a dip-net. In 1939 and 1940, fish were captured in Departure bay by means of an electric light and a lift-net.

For the most part, vertebral columns were prepared for examination by maceration of the whole fish in thirty percent alcohol. The softened tissues were then teased away from the skeleton, and the backbones dried before examination. Where insufficient maceration made cleaning difficult, the vertebral columns were examined while wet by transmitted light under the binocular microscope. Counts on dried skeletons were made under the binocular by reflected light. Specimens of less than thirty millimetres length were found to be too delicate in structure for the foregoing treatment, and were stained and cleared by the alizarin method (Hollister 1934).

The vertebral count of an individual was considered as the number of vertebral centra occurring between, but not including, the basioccipital and the hypural bones. Backbones containing complex centra were considered as abnormal and no attempt was made to include them in the data.

ACKNOWLEDGMENTS

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SAMPLING

Since the conclusions drawn in the present paper are based on the demonstration of differences in vertebral count between samples, the extent and effect of selection in the sampling method must be considered. Tester (1937) demonstrated that in samples of adult herring the larger fish in a single year class tended to have higher vertebral counts. In a later section it will be shown that this is also true for herring in their first year. It follows that any selection on the basis of length will produce samples which are not random with respect to vertebral number.

Thompson (1917) reports a differential composition of schools of young herring, the smaller fish keeping to the upper layer of the school, and when close to shore being found also at the inshore edge. Observations made during the course of the present investigation tend to confirm these statements, and it has been found that samples taken with the dip-net (at the surface of the water) are generally of somewhat smaller average length than those taken by less

selective methods. While it is not argued that selection due to differential schooling is entirely absent, it is felt that the effect is at a minimum in samples collected with the seine, dynamite, or light. This is confirmed by the 1939 sampling in Departure bay, in which successive samples of two distinct groups of fish taken throughout the summer could be recognized not only by their differences in length composition, but also by their characteristic vertebral counts. Furthermore, as will be shown later, even if a sample is divided in two parts according to length, the difference in vertebral number will average only 0.16 vertebrae. Many of the differences between samples are considerably greater than this figure.

RESULTS

It will be observed in the vertebral data of table I that considerable variation may occur among the same year class in different localities, and between different year classes in the same locality.

Heterogeneity of the Vertebral Data

The Saltspring island population of adult herring, as described by Tester (1937), is presumably maintained by the addition of recruits from a large number of spawning and nursery grounds along the southeast coast of Vancouver island and elsewhere in the strait of Georgia. All the samples of immature fish on which the present paper is based were taken in this general area, and it is therefore desirable to know whether all could have been drawn from the same population. Applying Fisher's method of analysis of variance as used by Tester (1937), it was found that the observed F value (Snedecor 1934) was distinctly lower than that calculated at a probability of 0.01 in 1933, 1934, and 1937; and was considerably higher in 1932, 1935, 1936, 1938, and 1939 (table II). These results prove that in certain year classes at least, samples of young herring taken in the southeastern area tend to be heterogeneous as regards vertebral count.

Since mean vertebral count may be influenced by water temperature during the period of early development (Tester 1938), heterogeneity of the vertebral data in immature herring does not necessarily prove the existence of separate populations. Samples from the same locality in different years may differ widely in vertebral count, as shown by the data from Fulford harbour in table I. The value of 52.708 vertebrae for the 1936 sample is particularly striking, since it is distinctly higher than found in any adult population in British Columbia, and considerably above the average for Alaska herring.

TABLE I. Vertebral counts of samples of young herring from various localities

Locality	Year class	No.	Number of vertebrae								Mean	$S(x-\bar{x})^2$
			48	49	50	51	52	53	54	55		
Fulford har.	1932	322	1		5	81	183	48	4		51.879	176.28
Saanich arm	1932	376		1	6	75	218	73	2	1	51.973	197.73
Departure bay	1932	120			5	45	54	16			51.675	68.34
Nanoose bay	1932	131		1	4	25	75	24	2		51.939	81.51
Saanich arm	1933	49				9	31	7	2		52.041	23.92
Fulford har.	1933	152			1	31	93	26	1		51.967	64.84
Nanoose bay	1934	195			4	68	104	17	2		51.718	93.50
Departure bay	1934	273			8	97	146	20	2		51.674	128.00
Fulford har.	1935	210			2	59	133	15	1		51.781	75.92
Nanoose bay	1935	258			2	52	151	50	3		52.000	122.00
Nanoose bay	1936	261			2	71	155	33			51.839	105.24
Departure bay	1936	26		1	1	5	16	3			51.731	19.12
Patricia bay	1936	55					24	27	4		52.636	20.72
Fulford har.	1936	294				9	99	156	29	1	52.708	142.88
Ladysmith har.	1936	72				20	35	16	1		51.972	39.94
Fulford har.	1937	261		1	3	69	158	30			51.816	111.16
Ladysmith har.	1937	279	1		1	66	166	45			51.903	128.39
Departure bay	1937	144		1	1	35	88	19			51.854	63.93
Ganges har.	1938	269			3	81	159	26			51.773	105.17
Whaleboat pass	1938	214			5	59	131	19			51.766	86.32
Mill bay	1938	183			1	52	101	28	1		51.869	84.85
Cowichan bay	1938	100				14	51	33	2		52.230	49.71
Fulford har.	1938	259			2	57	149	48	3		51.973	124.81
Preedy har.	1938	282			7	81	169	25			51.752	116.62
Tod inlet	1938	41				18	17	5	1		51.732	24.05
Nanoose bay	1938	171			1	56	88	25	1		51.819	83.38
Deep bay	1939	197			3	39	118	37			51.959	87.67
Ladysmith har.	1939	31				7	20	4			51.903	10.71
Porlier pass	1939	126			1	29	86	10			51.833	39.50
Active pass	1939	295			3	62	176	53	1		51.956	130.43
Bidwell bay	1939	214			2	41	134	34	3		51.977	94.88
Departure bay	1939	104				42	58	4			51.635	32.13
" "	1939	653		1	8	209	373	60	2		51.749	277.00
" "	1939	559			3	138	344	68	6		51.885	234.68
Maple bay	1939	319			4	83	181	48	3		51.905	154.70
Fulford har.	1939	98			2	35	52	9			51.694	42.82
" "	1939	76				15	43	17	1		52.053	35.79
Bedwell har.	1939	188			1	53	112	21		1	51.835	81.89

TABLE II. Analysis of variance of vertebral counts in each year for young herring of several localities

Year class	Degrees of freedom		Mean squares		Observed F	Calculated F (P=0.01)
	Between	Within	Between	Within		
1932	3	945	2.806	0.554	5.06	3.80
1933	1	199	0.203	0.446	2.20	6352
1934	1	466	0.220	0.476	2.16	6361
1935	1	466	5.553	0.425	13.06	6.69
1936	4	703	31.550	0.466	67.70	3.35
1937	2	681	0.513	0.446	1.15	4.64
1938	7	1511	3.567	0.447	7.98	2.67
1939	11	2848	2.517	0.429	5.87	2.26

Samples from the same spawning ground in a single season may also differ significantly in mean vertebral number, as illustrated by the following data for post-larvae from three separate spawnings in Departure bay and two in Fulford harbour in 1939. Average water and air temperatures during the early developmental period are presented for Departure bay to illustrate the possible effect of environment in producing these differences.

	Mean vertebral count	D	$S.E._D$	$D/S.E._D$	Temperature (°C.) Water	Air
Departure bay:						
First spawning.....	51.885	0.136	0.0375	3.6	8.2	5.8
Second spawning.....	51.749	0.114	0.0676	1.7	8.4	8.2
Third spawning.....	51.635				10.9	9.5
Fulford harbour:						
First spawning.....	52.053	0.359	0.1033	3.5		
Second spawning.....	51.694					

On the other hand, a difference between the young fish from two localities, which tended to remain constant from year to year, and which persisted in the adult fish returning to spawn, might be expected to indicate a lack of free intermingling between the two areas in question. Tester (1937) admits the possibility of any so-called local population being divisible into two or more units. Evidence that the immature fish from Departure bay and Nanoose bay differ significantly from those taken in all other areas by reason of the high percentage of vertebral columns containing complex centra has been presented in a previous paper (McHugh 1942). Taking the mean number of vertebrae in Fulford harbour samples as an example, statistically significant differences can be shown to exist between this locality and Nanoose bay in 1935, 1936, and 1938, and between Fulford harbour and Departure bay in 1932, 1936, and 1939. The criterion of significance is a difference in average vertebral number at least 2.5 times as great as its own standard error. A study of the immature fish therefore shows that differences are present. Partial or complete segregation throughout their life would tend to preserve these differences.

Relation between Mean Vertebral Number and Date of Hatching

To determine the date of hatching from the average length of each sample, a knowledge of the growth rate was necessary. In a study of the rate of growth of young herring in Departure bay (McHugh 1940) it was found that during the first three months of life the increase in length approximated a straight line. This was found to be true of the fish from two separate spawnings in 1939, the only point of difference being a slightly higher growth rate in the later-hatched group. In the spring of 1940, weekly samples were again taken in Departure bay, and the growth in length was again found to approximate a straight line. The higher rate of growth in the spring of 1940 may have been associated with higher water temperatures, as shown by the following tabulation:

	Average length in millimetres		Smoothed water temperature (°C.)	
	1939	1940	1939	1940
At hatching.....	8.5	8.0	9.2	9.9
First week.....	11.0	10.5	9.8	10.3
Second week.....	13.0	13.0	10.2	10.8
Third week.....	15.0	17.0	10.7	11.2
Fourth week.....	17.5	20.5	11.2	11.7

Using an average curve to represent rate of growth, the approximate date of hatching of each sample of young herring was calculated from the average length of the sample at the time of capture. Since environmental conditions may vary considerably on the same dates in successive years, the data for each year were treated separately. In 1939, for example, by plotting the mean vertebral count against the calculated date of hatching for each sample, the dotted line shown in figure 1 was obtained by the method of least squares. Sufficient samples were not available for other years to permit the fitting of reasonably accurate lines, and the points for these are therefore merely indicated without comment. The data for 1939 show a statistically significant negative correlation between vertebral number and date of hatching ($r = -0.76$; $P < 0.01$), and the data for 1936 and 1938 approach significance. Treating the data for all years together, a significant negative correlation was obtained ($r = -0.569$; $P < 0.01$). The straight line fitted to these points is represented in figure 1 by an unbroken line.

Relation between Vertebral Number and Length

Dealing with mature herring, Tester (1937) has shown that a slight tendency is evident within a year class for the larger fish to have higher vertebral counts. The same tendency is evident within individual samples of herring in their first year. By arranging the data for each sample according to length, and dividing the resulting series into two equal parts, average vertebral counts for the large and the small fish were obtained (table III). All samples for which lengths and corresponding vertebral counts were available were included, omitting samples of less than one hundred individuals. The grand mean vertebral

count for the smaller fish is 51.79, and for the larger fish, 51.95 vertebrae. The difference (0.16 vertebrae) differs very significantly from zero ($P<0.01$).

By taking weekly samples in Departure bay it has been possible to follow the progeny of a single spawning throughout the first few months of development. Most of the individual samples taken in other localities correspond closely in length composition to that of a homogeneous group, and it is therefore probable that the majority contain fish from a single spawning. Hubbs (1925) is of the opinion that the larger fish developed earlier in the spring, were subject to lower temperatures, and therefore have higher counts. In a sample originating from one spawning the effect of temperature in producing variation must be at a minimum, if not entirely eliminated, since all the eggs are deposited within a short space of time and hatch in much the same order. Under these circum-

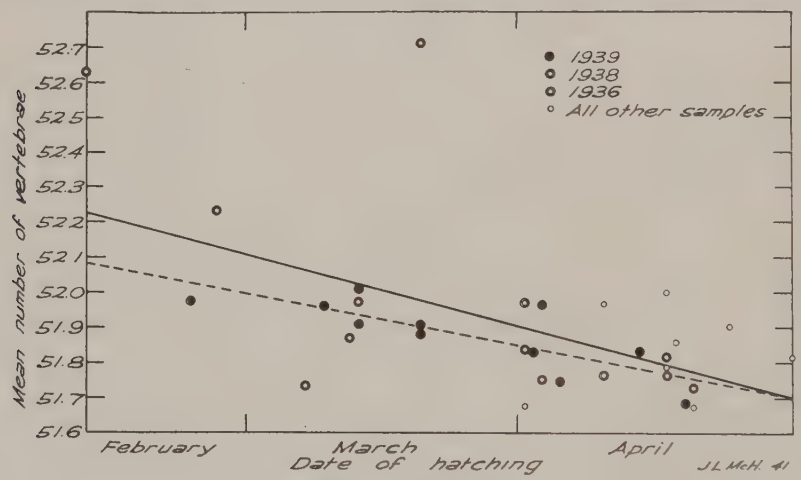

FIGURE 1. Relation between mean number of vertebrae and calculated date of hatching for all samples. ————, all samples; – – – – – – 1939 samples.

TABLE III. Comparison of mean vertebral counts of small and large fish in several samples

Locality	Year	Average count of length groups		
		Small	Large	Difference
Nanoose bay	1934	51.66	51.80	+0.14
Fulford har.	1935	51.71	51.88	+0.17
Fulford har.	1937	51.69	51.92	+0.23
Ladysmith har.	1937	51.83	51.98	+0.15
Preedy har.	1938	51.72	51.82	+0.10
Fulford har.	1938	51.91	52.12	+0.21
Active pass	1939	51.90	52.01	+0.11
Bidwell bay	1939	51.88	52.09	+0.21
Maple bay	1939	51.84	51.93	+0.09
Grand mean:		51.79	51.95	+0.16

stances it would appear that the correlation between length and vertebral count could not be explained on the basis of temperature, but must be associated with a factor such as difference in rate of development. Thompson (1917) suggests a somewhat similar interpretation of the phenomenon.

Abdominal, Caudal, and Tail Vertebrae

The anterior vertebrae with haemal arches open are known as abdominal vertebrae. Those posterior with haemal arches transversely bridged are termed caudal vertebrae. In the last few vertebrae preceding the urostyle the ventral processes are joined to the centrum by two separate attachments, as illustrated by Ford (1933). Following Ford's nomenclature, these are referred to as tail vertebrae.

Variation in Abdominal and Caudal Count between Samples

Working with vertebral columns from adult fish, Tester (1937) has found that the variation in vertebral count between localities is confined to the abdominal portion of the backbone. He shows that the mean caudal count does not increase in samples with mean total counts ranging from 51.85 to 52.25 vertebrae. In young Pacific herring, much the same relationship exists between abdominal and caudal counts, as shown by the following tabulation:

Locality	Year	Mean number of vertebrae		
		Total	Abdominal	Caudal
Departure bay	1934	51.67	22.94	28.73
Tod inlet	1938	51.73	22.93	28.80
Preedy harbour	1938	51.75	23.04	28.71
Nanoose bay	1936	51.84	22.99	28.85
Departure bay	1937	51.85	23.10	28.75
Ladysmith harbour	1936	51.97	23.32	28.65
Nanoose bay	1935	52.00	23.25	28.75
Fulford harbour	1938	52.02	23.17	28.85
Patricia bay	1936	52.64	23.22	29.42
Fulford harbour	1936	52.71	23.53	29.18

In the last two samples listed, the caudal counts are higher than might be expected. This may be associated with the unusual conditions which produced the exceptionally high total counts in 1936. Eliminating these two samples, the coefficient of correlation was calculated for both abdominal and caudal count with total count. Between abdominal and total count, a strong positive correlation of high statistical significance was obtained ($r = +0.88$; $P < 0.01$), while for the caudal count a weak correlation of no significance was obtained with total count ($r = +0.083$; $P < 0.8$). In mean total counts ranging from 51.67 to 52.02, differences are therefore confined almost entirely to the abdominal region of the backbone. Tester (1937) found abdominal count even more useful than total count in some cases, and thereby demonstrated a difference between Barkley sound and Saltspring island where no significant difference in total count existed.

Variation in Abdominal and Caudal Count within Samples

Within a sample, however, an increase in the total number of vertebrae seems to be more equally distributed between the abdominal and caudal regions. In illustration of this point, the average abdominal and caudal counts for individuals with total counts of 51, 52, 53, and 54 have been calculated for several samples (table IV). The ratio of abdominal count to total count for individual fish is about 0.445, and therefore to increase proportionally the abdominal count should increase by 0.445 for each addition of one vertebra to the total count. The average increase within samples is only 0.35 vertebrae, indicating that increase of vertebral count within a sample is reflected to a greater extent in the caudal region.

Table IV. Mean abdominal and caudal vertebral counts for individuals having different total counts

Locality	Year	Number of abdominal vertebrae for total count of:				Number of caudal vertebrae for total count of:			
		51	52	53	54	51	52	53	54
Departure bay....	1934	22.68	23.10	23.55		28.32	28.90	29.45	
Nanoose bay.....	1935	22.90	23.32	23.36		28.10	28.68	29.64	
Nanoose bay.....	1936	22.75	23.04	23.70		28.25	28.96	29.30	
Ladysmith har....	1936	23.05	23.31	23.69		27.95	28.69	29.31	
Fulford har.....	1936		23.28	23.63	23.90		28.72	29.37	30.10
Patricia bay.....	1936		23.08	23.26	23.75		28.92	29.74	30.25
Departure bay....	1937	22.60	23.16	23.79		28.40	28.84	29.21	
Fulford har.....	1938	22.89	23.13	23.54		28.11	28.87	29.46	
Preedy har.....	1938	22.92	23.01	23.67		28.08	28.99	29.33	
Tod inlet.....	1938	22.72	23.06	23.20		28.28	28.94	29.80	
Grand mean:		22.81	23.15	23.52	23.82	28.19	28.85	29.48	30.18
Difference in:									
Total count.....			1.00	1.00	1.00		1.00	1.00	1.00
Abdominal count			0.34	0.37	0.30				
Caudal count ...							0.66	0.63	0.70

Since all the individuals in a sample of young herring probably developed under almost identical conditions, the effect of temperature and the environment in general can be ignored. Thus the increase in caudal count with total count within a sample must be explained on the basis of a factor such as inherent developmental rate, which is not affected by external conditions. However, the values for caudal count vary about the same mean under almost any set of environmental conditions, and the effect of environment therefore shows itself as a change in the mean number of abdominal vertebrae.

Variation in Tail Count

Ford (1933) found for Atlantic herring that individuals having a higher total count had a disproportionately larger number of tail vertebrae. In young Pacific herring this is true within samples (table V), the increase in tail count

when the total count increases by one vertebra being 0.23 vertebrae. If the proportions were equal, an increase in total count of one would be followed by a gain of approximately 0.1 in tail count.

Between samples, however, the mean tail counts appear to be more erratic, and differences may be found which are not reflected in the total count. Comparing the 1934 year class in Nanoose bay with the 1935 year class in Fulford harbour, no significant difference is evident in total count. The difference in mean tail count, however, is 5.9 times its own standard error, a difference which is highly significant statistically.

TABLE V. Mean number of tail vertebrae for individuals having different total counts

Locality	Year	Count	Count	Number of tail vertebrae for total count of: 51	52	53
Nanoose bay	1934	51.72	5.61	5.40	5.74	
Fulford har	1935	51.78	5.21	5.02	5.26	5.47
Fulford har	1937	51.82	5.07	4.98	5.07	5.37
Ladysmith har	1937	51.90	5.04	4.94	5.26	5.52
Cowichan bay	1938	52.23	5.30		5.14	5.37
Grand mean:		51.95	5.15	4.98	5.18	5.43
Difference in:						
Total count					1.00	1.00
Tail count					0.20	0.25

SUMMARY AND CONCLUSIONS

Statistical treatment of data from samples of young herring collected in waters along the southeast coast of Vancouver island demonstrates heterogeneity in the vertebral material. It is pointed out that this does not necessarily indicate the existence of more than one population in this area, since mean vertebral number is known to be affected by environmental conditions during the period of early development, and free intermingling of the schools in later life would cause such differences to disappear.

Certain evidence has been brought forth suggesting a lack of complete intermingling among the adults supporting the commercial fishery in the southeastern area. Statistically significant differences in mean vertebral number which tend to remain constant from year to year can be demonstrated between the young from different spawning grounds. Partial segregation of these groups throughout their life would tend to preserve such differences. In a fishery such as that carried out in the Saltspring island area, where many of these groups are undoubtedly exploited, it is easy to see how a lack of complete intermingling would increase the difficulty of sampling the fishery.

On calculating the approximate date of hatching by means of an average growth curve obtained from weekly samples taken in Departure bay, a significant negative correlation was established between date of hatching and mean verte-

bral number, suggesting that groups hatched earliest have the highest vertebral counts.

Within a sample, the largest fish tend to have a higher number of vertebrae. Since it is likely that many of the samples examined are the result of a single spawning, the individuals in each must have developed under almost identical environmental conditions. It is therefore more probable that the phenomenon is caused by differences in inherent developmental rate than by environmental factors.

As in the adults, the variation in mean total count between localities is made up almost entirely of a difference in the mean abdominal vertebral count. Within a sample, however, both abdominal and caudal counts vary, and an increase in total count produces a disproportionately large gain in mean caudal count. This must also be an effect of variation in inherent developmental rate, since the caudal count varies about the same mean in almost all samples.

A disproportionate rise in mean tail vertebrae in individuals having higher total counts has been observed within a sample. Between samples, the tail count may differ significantly where no difference in total count exists, and it is thus possible that this character might be used in separating populations.

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Death of Salmon and Trout with High Temperature

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ABSTRACT

Deaths in Moser river, N.S., in 1939 were at time of day, on days, and in locality with highest water temperature. Symptoms were loss of sensitivity to light and very rapid breathing with recovery therefrom in cool water; otherwise followed by loss of equilibrium and cessation of breathing with death. Freshly run grilse died at about 29.5°C. and acclimated grilse at about 30.5°. Large salmon died first and parr survived. In experiment, the latter died between 32.9 and 33.8°, the smallest at the highest point. Death is considered due directly to temperature.

Rainfall after long period of drought brought the salmon from the sea into the river to die where water was shallowest and without cascades, therefore warmest. The very high temperatures and deaths occurred in afternoon from sun's heat and at ends of periods of days with moist air from the ocean and mounting water temperatures, dew point temperatures and mimimal air temperatures.

Prevention is seen possible by bringing fish in early with artificial freshets, by constructing low dams to create deep pools and cascades, by making a deep channel or by raising level at critical periods with stored water.

On a survey of salmon streams in Nova Scotia in 1938, it was learned that salmon had died in the St. Mary and Moser rivers, on the outer coast between Halifax and Canso, in the summer of 1937 at a time when those rivers were very low. Selection of the latter river for intensive work on the salmon and the extreme local drought of 1939 permitted observation of the phenomenon. The principal questions to be answered were: (1) what was the character of the death of the salmon? (2) What were the circumstances of time and place as indicating possible causal factors? (3) Would the related conditions result in death in experiment? (4) How were these conditions brought about in the river, and could they be prevented from developing?

LATE AUGUST, 1939 IN MOSER RIVER

From August 19 to 23 the trap in operation about 1½ km. (1 mile) above the head of tide had failed to show any upward movement of salmon, although some were known to be in pools just below the trap and also near the highway bridge not far above the head of tide. At the latter place they were in evidence near the surface around midday on August 23, so that boys and men with previous experience expected them to die and began to stone them.

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Deaths on August 24

Shortly after noon stoning of the salmon at the bridge began, but was soon stopped by the local guardian. At 3.30 p.m. the first dying fish was seen and picked up below the bridge, proving to be a two-sea-year salmon, 71 cm. long. At various times until about 5.30 p.m. six grilse, from 52 to 56 cm. long, were taken from the water within 0.6 km. (1/3 mile) of the head of tide. All seven fish were females, seemingly in good condition, and were discovered floating or stranded in shallow water with more or less of the white, ventral surface showing, which indicated loss of control of equilibrium.

Behaviour of Salmon

relation to cool water

Examination of the river on August 24, after the first dead fish was found, revealed grilse congregated only in two places—five to seven of them in a shallow side arm of the river below the bridge, and three in another side arm above the bridge, both on the west side. At 4.20 p.m. temperatures were 30.4 to 30.6° C. in the running water, 29.7° at the head of the side arm above the bridge and 28° in the side arm below the bridge where most fish were congregated. The latter arm was as low as 26.1° at its head, and the coolness of these arms was quite evidently due to spring water from the gravel bed of the river, which is on that side only.

Parr as well as grilse were congregated in these cool side arms. On his way up river that afternoon, Mr. A. C. Nicol observed something new—numerous parr along the west shore below Mill brook, whose water, coming from Mill lake, is during the day cooler than the river water.

activity

A grilse was observed to ascend the rapids above the bridge about 4.00 p.m., an unusual event during the day. The trap for ascending fish, when examined at 9.00 p.m., contained 1 grilse and 29 parr. There had been no grilse for some days and never before had there been more than an occasional parr in the trap (Mr. H. C. White, who has operated it, states that there are perhaps half-a-dozen in a whole summer). Clearly, the adult salmon were moving at a time of day when they are ordinarily quiet, and the parr, which migrate but little at any time were moving somewhat extensively and in large numbers.

The salmon that were congregated in shallow water rested quietly, and apparently quiescence preceded collapse. Breathing was rapid, 120 to 140 respirations per minute. Individual salmon, seemingly somewhat recovered in the cooler water of the side arm, became active and swam out only to return in a short time. As the temperature of the river dropped in the late afternoon they failed to return and recovery was apparently complete.

sensitivity to light

Salmon are quite sensitive to light, dashing about when stimulated by moving shadows and avoiding strong daylight by keeping in deep water. The larger the individual the deeper the water to which it confines itself.

On this occasion, the grilse were in such shallow water that in some the upper part of the caudal fin, all of the dorsal fin and part of the back were above the surface of the water. The parr were also in shallow water, but deeper than the grilse and the smallest (a yearling?) was deepest and probably at about its ordinary depth. Therefore, the normal vertical distribution in sunlight according to size was reversed.

The grilse were mostly insensitive to light, waving one's arm over them not producing the slightest reaction. The parr were definitely reactive and the smallest most so. The grilse in the cooler side arm showed recovery by becoming again sensitive to light as tested by waving one's arm.

Death on August 26

No moribund or dead salmon were found on August 25, but on August 26, in the afternoon, several grilse entered one of the cool side arms near the bridge, but did not reach very shallow water nor remain long, and no parr were in evidence. One salmon was found in a dying condition, a male grilse in shallow water near shore at the river mouth (head of tide at low water). Its finder believed it to have been affected by entering the warm river water, which it might do abruptly at this place.

EARLY AUGUST, 1939 IN MOSER RIVER

Other members of the investigational staff at Moser river reported the following: For the first five days of August no salmon ascended through the trap, but they were very numerous in the upper part of the estuary and seen jumping out of the water, as many as three being in the air simultaneously. On August 5, many were at the very head of tide and somewhat above, some in shallow water among the stones with dorsal and caudal fins projecting. On August 6 they were farther up the river, some (23) at the trap by noon, and in evidence at the surface and ascending rapids even at noon. Some commenced to die at 1.00 p.m. and deaths continued until 4.00 p.m. at least, from 35 to 50 (nearly all grilse) being picked up in a dead or dying condition. At least 14 more were found dead at various times during the following fortnight and at various places from the upper part of the estuary to Rocky island, 3½ km. (2¼ miles) up river in the cascade section. Most of the deaths on August 6 were near the highway bridge, where at 1.30 p.m. the water temperature was 29.4°, but they occurred all the way up to the trap, where the temperature at 2.15 p.m. was 29.2°C. The temperature did not again rise so high for over a week.

Not only the observed movements of the fish, but also the large numbers of sea lice on them showed that on August 6 they were freshly in from the sea. Deaths on that day were not correlated with damage by the lice, although some had the epithelium near the head extensively removed ("white heads"). Later, however, the dead or dying fish found at various places had even the reddish muscles exposed ("red heads"). At least a few of the fish observed on August 6, made spasmodic rushes, sometimes on the shore, before death.

IN PREVIOUS YEARS IN MOSER RIVER

According to local reports, in 1937, with very low water and many fish in tidal water, grilse were on one occasion numerous in the pool at the bridge and some became weak or died, from 50 to 75 being taken out. Most were

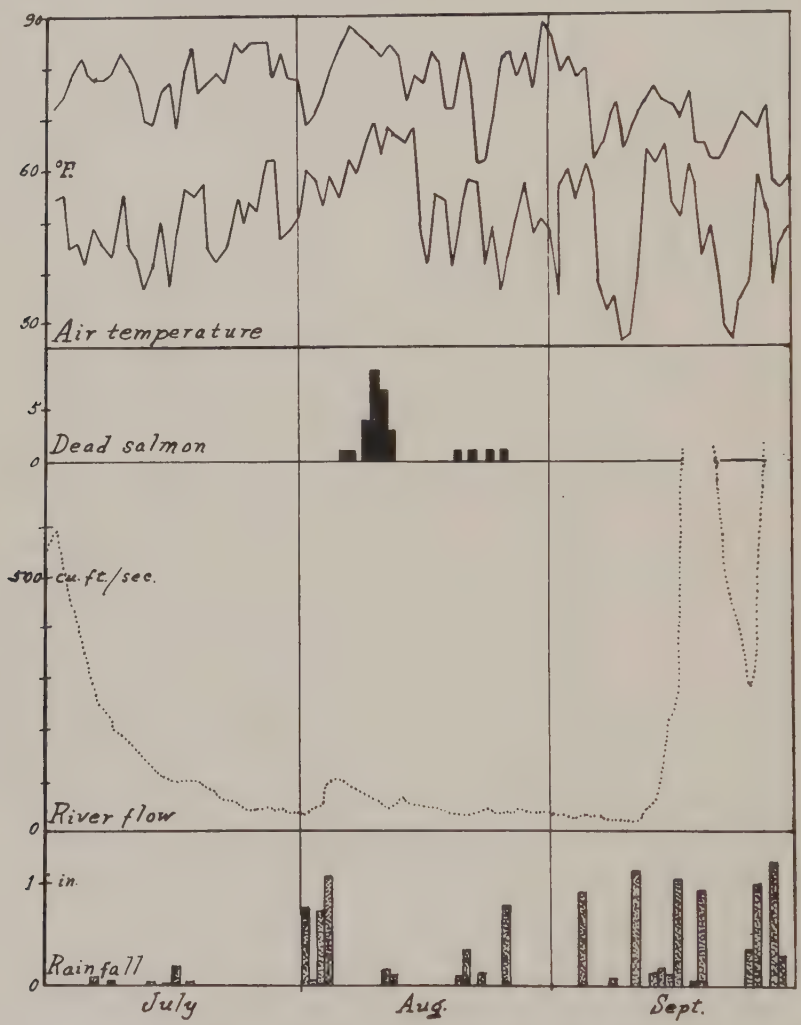

FIGURE 1. Rainfall, river flow, and maximal and minimal daily air temperatures at the St. Mary river, N.S. in July, August and September of 1937, in relation to death of salmon as reported by Inspector Manson. From records of the Dominion Meteorological service and of the Dominion Water and Power Bureau, taken at Stillwater.

removed on the first day and the condition did not last a week. Some had whitish heads. They were found mainly at the bridge and the limits were from a short distance below the bridge to Beaver hole, 3¼ km. (2 miles) upriver in the cascade section.

It was also reported that salmon had died in the river during the very dry season of 1921. At Stillwater, in June, July and August of 1921, the rainfall was 6.70 inches (17.0 cm.), as compared with an average of 12.77 inches (32.4 cm.) for 22 years' observation. Perhaps more significant was a period of practically four weeks of almost absolute drought preceding moderate rains on July 15 and 20.

IN 1937 IN ST. MARY RIVER

Salmon, as reported by Inspector M. M. Manson and Dr. L. M. Silver, died mainly in early August in the middle of a period of low water lasting from mid-July almost to mid-September (fig. 1). This was not, however, when the water was lowest, but following the heaviest rainfall of the dry period, as if involving salmon newly brought in from the sea by the freshet. Most of the deaths and the first deaths were in and near the Silver pool, at the junction of the East and West rivers, about 15 km. (10 miles) above the head of tide, but at the last they were as far down as Sherbrooke near tidal water, and as far up as Caledonia, about 24 km. (15 miles) up the West river. The pool contained very many salmon ("hundreds") and its temperature reached levels ranging from 82° to 84°F. (Inspector Manson) and on one day was 77° in the morning and 85°F. (29.5°C.) at night (Dr. Silver). While its lower end was traversed by the fair flow of the West river, the flow through it from end to end from the East river was reduced to a mere trickle coming from the Glenelg lakes through a weedy, sluggish channel. The deaths were chiefly of large salmon of about 20 lb. (9.07 kg.) weight, which were doubtless three-sea-year fish, and no dead grilse were found, although there were many in the river.

MORTALITY OF TROUT

The local trout (*Salvelinus fontinalis*) were ascending the Moser river from the sea in early August of 1939, though in small numbers (last of the "run"). The only dead fish reported were three in the trap on the morning of August 2, and on the previous day the water temperature at the trap was the highest (29.5°C.) that it reached before the run ceased. We were informed that in 1933 or 1934 the ranger for the Game Sanctuary who travelled up and down the Moser river system, reported that trout were congregated in all the springs and that some died in both Kelly and Middle Chain lakes.

CAUSAL FACTORS

Lepeophtheirus

In early August of 1939, the salmon were heavily infested with sea lice, which in extreme cases had removed much of the epithelium, chiefly in the occipital region. There seems no question that this condition resulted in the death of quite a number of the salmon (White 1940), perhaps only after August 6, when the chief mortality occurred, and when no extreme cases of damage from the lice were yet observed. Doubtless death would result from the action of

the salt or fresh water on the tissues unprotected by epithelium and through them on the composition of the body fluids (Marshall 1931). Death would be expected to occur wherever the salmon happened to be and over a longish period of time. Reports were not very precise, but, of the 14 reported as found dead from August 8 to 19, at least 10 were stated to have been badly damaged, and they ranged from the estuary to Rocky island; there may well have been dead salmon farther up the river, since there would be little chance of their being observed. These deaths, seem to have been due indirectly to the lice, which do not remain long on fish that have entered freshwater.

Asphyxia

The only instance where lack of oxygen seems likely to have been a factor in the mortality is that of the St. Mary river, where very many salmon were congregated in a pool, through the greater part of which very little water was running. But that they died from asphyxia rather than high temperature is not at all clear.

The deaths in Moser river at the bridge in 1937 and on August 6, 1939, are reported to have been associated in some cases with convulsive dashes, which would indicate asphyxia. There is not likely to have been any lack of oxygen with such an amount of running water, only temporarily heated. The fish were stoned and chased about in efforts to capture them, and there may well have been individuals asphyxiated by overexertion (Huntsman 1938).

High Temperature

That the deaths of salmon on August 6, 24 and 26, 1939 occurred in the afternoon makes them coincide with or follow the highest water temperature of the day, and those also were days of highest temperature (fig. 2). High temperatures were recorded also on July 26, 29, 31 and August 1 without any deaths being reported, but they were not as high as that of August 24, the only day on which salmon not newly in from the sea died and when no fish seemed to be entering the river. The deaths occurred in August, 1939, the month and the year with highest temperature (table II). In 1940 the temperature did not go above 29½° as recorded by the thermograph and no deaths were reported, but on the day in July when it was highest (29°C.) one grilse was observed by Dr. W. S. Hoar and Mr. A. C. Nicol in shallow water at the bridge (3 or 4 in somewhat shallow water in mid-channel) and young salmon were congregated in shallow cool water (27.3°) at the mouth of a brook. In 1941, not even as high a temperature as 27° was recorded after June, and neither deaths nor congregation of fish in shallow water were reported.

The part of the river where most salmon died, a short distance above the head of tide near the bridge, seems to reach a higher temperature than any of the connected waters. The limited data available (fig. 3) indicate that near the bridge on August 24, 26 and 27 the high temperature developed later, was somewhat more pronounced and passed away more slowly than at the trap. Continuous records at both places from August 29 to September 4 (fig. 3) show that the relation varies, delayed afternoon fall in temperature at the bridge

being the most constant. Also the water in the lowland section (from the head of tide to just above the trap) gets warmer than (1) the *cascade section* above it [2.15–2.50 p.m., Sept. 13, 1939: above bridge, 23.2°; below trap, 21.3–22.1°;

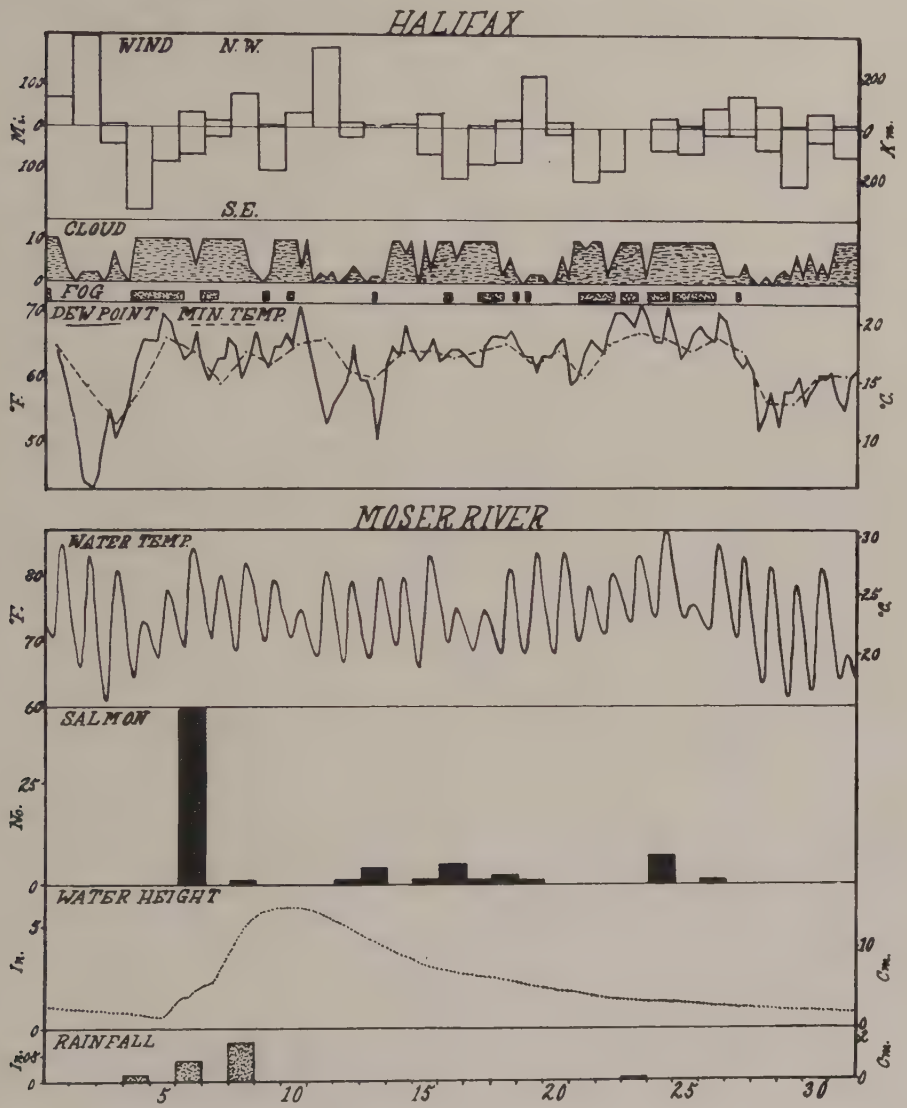

FIGURE 2. Rainfall, water height and water temperature in relation to death of salmon at the Moser river, N.S. in August of 1939. Also (above) dew point, maxima for air temperature, fog, cloudiness and daily mileage for offshore (northwest) and onshore (southeast) winds as recorded at Halifax by the Dominion Meteorological service.

above first Oak island, 21.2–21.4°; Little Gaspereaux falls, 20.3–20.5°], (2) *Mill lake* and *brook* emptying into it below the trap [1.15–1.45 p.m., July 25, 1939: above bridge 27.4°; below trap 27.6°; brook, 25.7°; 30 m. (100 ft.) offshore in

lake, 24.8° at surface and 23° at 1 m. (3¼ ft.) down], and (3) the *estuary*. Continuous records at the trap in the lowland section and at Rocky island in the cascade section from September 4 to 9 (fig. 3) show lower maxima and higher minima in the cascade section.

The deaths reported over an ill-defined period around 10 days after the principal mortalities in the St. Mary river in 1937 (fig. 1) and in the Moser river in 1939 (fig. 2) were not associated with high temperature. While injury

FIGURE 3. Course of water temperature in 1939 in the Moser river, N.S. Upper,—from August

P. 492. Erratum: In figure 3, lower graph, dotted line is for the river at Rocky island, and interrupted line at the fish trap (not as given in the legend).

in the cascade section. Continuous line,—at the highway bridge; dotted line,—at the fish trap; interrupted line,—at Rocky island.

from sea lice may have been a factor there is a fair probability that some of the fish had been weakened by exposure to the high temperature and recovered only to die later.

SPECIES

Trout, which are ordinarily related to cooler water than are salmon, would be expected to be more affected by high temperatures, but, even when ascending the river in late July and early August, they were found dead only on one occasion. However, we do not know that they remained during the heat of the

day where the water became so warm, nor to what extent size and acclimatization may have been involved. Their habit of congregating where cool spring water issues is well known. The salmon, although showing some tendency to enter the cooler water of the side arms with the very high temperatures of August 24, were definitely ascending the river and dying in the warm water on August 6, with the cool water of the estuary (from which they had come) only a short distance away and down stream.

Size

Salmon larger than grilse are rare in the Moser river (4.5% in 486 passing through the trap from June to August inclusive in 1939). The only one known to be in the vicinity of the bridge on August 24, 1939, was the first fish to die, whereas of at least 12 grilse in the vicinity only 4 died and not a single parr. This evident increase in susceptibility to death with increase in size agrees with the described gradation with size in degree of loss of sensitivity to light at the high temperature. Also in the St. Mary river, most of the salmon that died were large salmon, some were of medium size, and none were small (grilse).

Knauthe (1895) found that, in pools left by streams that ceased to flow, the small individuals of trout (*Salmo fario*), perch (*Perca*), pike (*Esox*) and other species died at lower temperatures than did the large ones. It seems probable, however, that other factors than temperature were involved and responsible for this reverse order in death according to size, since the fish were crowded in stagnant pools, diminishing daily in size.

Acclimation

The grilse that died on August 6, 1939, were from a lot that had been seen migrating up from the estuary on that day or the previous evening and they died with a maximum temperature between 29 and 29½°C. The grilse found dying at the river mouth on August 26 had numerous sea lice (*Lepeophtheirus salmonis*) on its skin, but was not perceptibly injured by them. To judge from this condition as well as from the place of finding, this fish was newly in from the sea. The salmon are said to reach the river mouth with rising tide, and this fish was found about 4.00 p.m., with high tide at 5.45 p.m. The temperature of the water had nearly reached 29.5°, and had been above 29° for several hours.

All large salmon and grilse either found dead or dying or closely observed alive in the shallow water on August 24 were without sea lice as far as could be seen. Three of the dead fish had some of the skin in the occipital region gone, which was evidence of their having had many sea lice at an earlier time. A grilse, dying at the bridge, bore a tag which had been fastened to it at the trap 51 days previously. It seems certain that all those dying on this day had been in the river for some time. For four days there had been no evidence of any movement of the fish as revealed by the trap, and on three days the water temperature at the trap had risen above 28°. The fish had had an opportunity to become acclimated. The water temperature on August 24 reached 30.5°

even at the trap and at the bridge it was above 30.5° at 4.15 p.m. Even so, not more than a third of the grilse near the bridge died. None of those left died on August 26 and they only approached the less cool side arm and did not enter the cooler one.

It is clear that there was definite acclimation to the high temperatures, as has been shown for various fishes (Fry *et al.* 1941).

EXPERIMENTS

It was not feasible to experiment with adult salmon, but only with small fish all of which had withstood the high temperatures. These were taken from the river on August 28, 1939, and the water in which they were held was warmed at the rate of 1°C. every five minutes. The dying period, as recorded, began with a decrease in swimming activity, shortly after which the fish lost their equilibrium (turned over), and ended with cessation of both swimming and respiration. Ages, numbers, lengths and dying periods of the salmon parr were as follows: 2+yrs., 1, 16.0 cm., 32.9–33.2°; 1+yrs., 1, 12.7 cm., 33.5–33.7°; 0+yrs., 5, 5.7–6.3 cm., 33.6–33.8°. Of young suckers (*Catostomus commersonii*), they were 1+yrs., 2, 4.2 and 4.8 cm., 35.1–36.1°.

In agreement with the mortality in the river on August 24, these young salmon that, although somewhat affected, had survived the high river temperatures died at temperatures 2° or more higher than had been reached in the river, and the smaller the fish, the higher the lethal temperature. The other species present withstood practically as high or higher temperatures. Davy (1862) and Day (1885) found fish to have lethal temperatures ranging from 80° to 100°F. (26.5–38°C.), Salmonidae having the lowest. Embody (1922) found that brook, brown and steelhead trout died between 84.2° and 87°F. (29–30.5°C.). Rushton (1926), however, found that 80% of his salmon and brown trout fry died with temperatures no higher than 25°C. Huntsman and Sparks (1924) obtained lethal temperatures ranging from 22 to more than 42°C. for various fishes, with the young *Gasterosteus* more resistant than the adults and with *Pseudopleuronectes* showing lower lethal temperatures with increase in size.

Other experiments were performed on August 8, 1940, in a season with much higher water and not so high water temperatures as in 1939. Dying periods for salmon parr were: 4 from Moser river near bridge, 31½–32½°; 1 from Holman's brook, 31.1–31.2°. Dying periods for trout were: silvery "sea" trout from Mill lake, 29–29½°; "brook" trout from Holman's brook, 30–30½°. Mill lake has cool bottom water; Holman's brook has warmer water; and Moser river has the warmest water.

The facts indicate (1) that resistance of salmon parr to high temperatures was higher for Moser river in the warm summer of 1939 than in 1940, and higher for that river than for the cooler Holman's brook, (2) that resistance of trout was higher for those newly in from the sea than for those living in a somewhat warm brook.

MECHANISM OF DEATH PROCESS

Where lack of oxygen in the water has caused death, the respiratory centre of the fish will have failed, stopping the breathing, after which other tissues will have disintegrated in the order of their ability to endure low tension of oxygen or subsidiary changes. Where overexertion has caused death, the lactic acid set free in the blood from the muscles lowers its oxygen-carrying capacity, and asphyxia, as just described, supervenes. In death through removal of the skin by the sea lice, the salt content of the blood becomes altered, which will in some way kill the various tissues.

For the most part, the deaths were from high temperature, in which there is primarily "failure of some coordinating mechanism (presumably in the higher nerve centres)" (Battle 1929). The cause of such death of the animal has been considered (Cameron 1930) to be "some damage to its coordinating mechanism, of which the underlying chemical or physico-chemical change cannot yet be stated" and death itself to be for the biochemist the cessation of the chemical changes and the physicochemical processes of life. Doubtless permanent cessation is to be understood, in order to exclude "suspended animation" in the cases in which no life processes have been detected. This aspect or conception of death as the permanent cessation of function is physiological. The other aspect of death is the change in the character of the organism, that is, in its structure, which is a morphological or chemical conception of death and agrees with Claude Bernard's definition,—"la mort est destruction organique." In this view, death is when the machine is broken beyond repair, so that it will not work again. If it happened not to have been working for some time before it was broken, death would not be when it stopped working, but when it was broken. On this basis, death is "the break down of the essential or specific structure" (Huntsman 1926), which recognizes that the processes of the particular life depend upon its specific structure and that the latter is repaired only when partly broken down, which to a greater or less degree is occurring rather constantly.

In death from high temperature, the "failure of some coordinating mechanism" may well indicate a break down in the structure of central nervous tissue or at least part of it, since Cameron and Brownlee (1915) found it to be functioning in part. In muscle, increase in opacity occurs as, with rising temperature, ability to function is lost, with final inability to recover (Battle 1926). This indicates coagulation (irreversible denaturation or break down) of the protein complex as the lethal effect. The temperature at which death occurs varies with the kind of muscle or other tissue, or even with the part of the cell (body of nerve cell, nerve fibre and myoneural junction) and is lowest in nerve cells and in the body of the nerve cell. There are also probably differences between different types of nerve cells (Battle 1929). Death of the whole animal, that is somatic death, from rising temperature, may be said to follow death of the cells of nerve centres, which are the first to die and which coordinate activities of other parts of the body.

On exposure to high temperatures in nature, the fish recovers on lowering the temperature both from the condition of stimulated migration, which is the

first particular effect of the rising temperature, and from the condition of insensitivity to light, the second evident effect, so that in these no irreversible change or death of tissue can be said to have occurred. Loss of equilibrium, cessation of swimming and cessation of breathing occur at a higher temperature and closely together. From these, unless just as they develop, there is no recovery on lowering the temperature. Death of nerve cells can be said to have occurred.

FRESHLY-RUN FISH AND RAINFALL

The greatest mortality in the Moser river in 1939 was of fish freshly in from the sea, dying at a lower temperature than those acclimated. For such to die they need to enter at or just before a time with high temperature. With practically no rain for a fortnight, there were on August 4 large numbers of fish near the head of the estuary. The slight mid-day rain of that day stirred them up, but the upward migration did not progress far. In the following evening and night of August 5 a heavier rain fell and the migration gathered strength, giving large numbers throughout the lowland section by noon on August 6, when a high temperature was reached and killed many. On August 7 there was a still heavier rainfall, which gave a continued migration of considerable numbers of fish from August 8 to 16; but, with higher water and lower temperatures, there was no particular mortality. On the St. Mary river in 1937, there was very little rain during July and none for the last 13 days. Then, rather heavy rain fell on the first four days of August, which would cause a big upward migration of salmon. The high water temperatures, as judged by minimal air temperatures, were apparently not reached until about August 9, by which time the fish were doubtless well upstream, as shown by the mortality occurring at about that time and over 9 miles (14.4 p.m.) above the head of tide. Since the Moser river rainfall seems to be quite similar to that of St. Mary river, doubtless the mortality on the former river in 1937 took place under similar circumstances but data are lacking. Thus a rather heavy rainfall following a rather long period of drought in summer is a factor in causing mortality.

CAUSES FOR HIGH WATER TEMPERATURES

Hydrographic

The salmon deaths all occurred during periods of very low water, which resulted in rapid rises of temperature on bright days with a high sun.

The lowland section of the Moser river, to which the mortalities were practically confined, both in 1937 and 1939 (only one reported elsewhere in each year), is broad, shallow and *without cascades* as compared with the cascade section above it. Observations on August 28, 1939, showed temperatures at two places in the lowland section to be 27.1–27.6° and 26.8–27.1° at 1.32 and 1.45 p.m.; and 27.4–27.6° and 27.3° at 3.30 and 3.22 p.m. From 2.05 to 2.45 p.m., temperatures above and below several of the cascades in the cascade section were (1) 25.5–25.7 and 25.0–25.4°, (2) 25.0–25.7 and 25.2–25.3°; (3)

25.4 and 25.1° and (4) 25.8 and 25.2°. This shows lower maximal temperatures in the cascade section (see also fig. 3) and lower temperatures below falls than above them. That falling or splashing water will become significantly cooler in dry air is well known. Tests made at Toronto gave in February with inside temperature of 22° a drop in temperature of 0.2 and 0.3° for water of 26 to 33° falling from 35 to 45 cm. The drop will depend upon the relative amount of water evaporated.

Perhaps the most important factor for high temperature is *shallow water.* Mill lake served to demonstrate this, since it is of variable depth, and contains such a luxuriant growth of aquatic plants (of which *Utricularia* is the most abundant) that there is but very slow, circulation of the water.

As seen in table I, the surface temperature of the lake at the afternoon peak period is lowest where the water is deepest and highest where it is shallowest, while at daybreak, the time of lowest temperature, the condition is reversed. As compared with the shallow river, the lake neither warms as much by day nor cools as much by night. Consequently the lake discharges into the river

TABLE I. Surface temperatures (in °C.) when maximal (early afternoon) and when minimal (early morning) on July 25 and 26, 1939, over various depths in Mill lake and also in outlet brook and in lowland section of Moser river, into which the brook discharges

	Mill lake			Mill lake brook		Moser river at trap	
Depth of water (m.)	1.5	1	0.5	at trap	at mouth	west side	east side
1.30–2.15 p.m.	24.8	25.7	25.9	25.5	26.1	27.6	27.6
4.30–5.15 a.m.	22.5		22.1	21.8	21.3	21.0	21.0

through Mill brook (table I) water, that in the afternoon is cooler and in the early morning is warmer than the river water. Doubtless, when the level of the water of the river is high, its temperature will be more like that of the lake, showing less fluctuation each day. This effect cannot be readily demonstrated since the daily heating and cooling of the water are so variable, depending upon

TABLE II. Highest monthly temperatures and coincident water heights (not comparable between years) in Moser river

Month	1939			1940			1941		
	Date	Temp. (°C.)	Height (in.)	Date	Temp. (°C.)	Height (in.)	Date	Temp. (°C.)	Height
June	21	24½	5½	18	21⅔	8	22	27¾	9
July	29	29¼	1	28	29	6	24	26⅔	12½
August	24	30½	<1	17	29½	3	9	24	15½

the weather. However, the summers of 1939, 1940 and 1941 were in that order as to water height from low in 1939 to high in 1941; and the highest river

temperatures (lowland section) recorded for the three summer months in each year show a general inverse relation to water height (table II), both within a particular year and between years.

Meteorological

Rainfall and river height

The height of the river is determined by rainfall and the summer of 1939 was exceptionally dry. The wells of Moser River village were regarded as lower than in 1921, the previous year of record low water within memory.

That slight rainfall acting through low river level was a factor for the mortalities is shown by the data for summer rainfall from 1936 to 1941 inclusive (table III) at Stillwater and Moser River. Where comparison is possible, the monthly rainfall is rather closely similar at the two places, and 1937 and 1939

Table III. Rainfall in inches (1 in.=2.54 cm.) at Stillwater and Moser River, N.S., during the summer months in recent years

	Stillwater (St. Mary river)						Moser River		
Year	1936	1937	1938	1939	1940	1941	1939	1940	1941
June	4.13	4.23	4.63	1.60	5.58	1.70	1.29	5.97	1.25
July	2.60	0.37	5.12	2.65	3.22	7.10	2.50	3.36	5.65
August	9.19	4.06	5.14	1.52	4.41	8.08	1.32	4.69	8.74

(the years of mortality) were dry years. The difference in July, 1941 (lower at Moser River) is explained by a heavy rainfall in July not occurring at Moser River village; however, it did occur upriver and produced a heavy flood in the river.

Moisture in the air

The high temperatures, which caused the deaths of the salmon, occurred on particular days. These were not the days with the clearest air, when the sun was most effective in raising the temperature of the water. On such days or series of days (fig. 2, August 1 to 3 and 27 to 29) both maximal and minimal water temperatures became lower from day to day. The high temperatures and the mortalities occurred at the ends of series of days (fig. 1, August 3 to 6 and 21 to 24) on which the daily rise in temperature was moderate, but with both maximum and minimum becoming higher from day to day. These days, as shown by the Halifax records, were characterized by onshore winds (southeast), fog, clouds, rising dew point, and rising minimal air temperatures. At Moser River the weather was "muggy," fairly clear and with fog working in from the sea in the afternoon or evening. Also, the mortality in the St. Mary river in 1937 occurred toward the end of a period with rising minimal daily air temperatures (fig. 1).

Mr. A. J. Connor of the Meteorological Service informs me that the records for 1939 show that old polar air present over the ocean off Nova Scotia and slowly

picking up moisture from the sea started on August 3 to move landwards. Conditions were unstable on August 4, but late the next day warmed continental air began to pass eastward, reaching Nova Scotia with a high dew point. Clear air on the morning of August 6 carried the temperature up from a high minimum. Similarly, on August 21 air from the ocean moved slowly landward, with a high dew point, and continued to do so during the following day. Early on August 23, warmed continental air with a high dew point replaced it, and this continued the next day, with rising temperature. In each case there was movement of air first from the ocean and then from the west. That from the ocean with its high dew point means air formerly dry, but with its lowest layers laden with moisture from the sea, while the movement from the west was of air which had attained high temperature and high vapour content during its stay along the Atlantic seaboard.

There seem to have been locally two important things: (1) a clear sky, at least during the day, to warm up the water; and (2) high moisture content of the air with fog particularly in the late afternoon and at night to prevent rapid loss of heat by the water. Under these conditions the temperature did not fluctuate greatly but rose steadily from day to day with increasing fluctuation, with the highest temperature on the final day. The warmer moist air resulting from the final movement of air from the west and southwest was doubtless a factor in reducing the loss of heat by radiation.

There is evidence that this effect was greater at the seaward end of the lowland section, that is, at the bridge as compared with at the trap. It seems to be not unusual (fig. 3, August 24, 26, 27, 29, 30 and 31) for the temperature at the bridge to reach its maximum later in the day than that at the trap. This would seem to be associated with higher moisture content of the air there, as was apparent from the distribution of the fog, and as would be expected from nearness to the sea.

Prevention

by early ascent of fish

Since the chief mortality in 1939 was of salmon that had been congregated for some considerable time near the head of the estuary and through lack of freshets had not ascended the river, the possibility of preventing mortality by early use of artificial freshets should be considered. Such freshets, produced at sufficiently short intervals, should prevent any large accumulation of fish near the head of the estuary. The facts indicate that they become heavily infested with sea lice in that location, which would be prevented by their early ascent. If they passed up the river with the freshets, they would not only have an opportunity to become acclimated to high temperature, but they would doubtless in large part pass upward beyond the lowland section, where alone there were fatally high temperatures.

by lower temperatures

Since the mortalities in Moser river have been practically confined to the

lowland section, which reaches the highest temperatures, measures to make this section more like the rest of the river might be efficacious in preventing mortality. The simplest course would seem to be *to construct low dams* across the river at suitable intervals. These would increase the depth of the water above each dam and the waterfall produced would be apt to excavate a pool below each dam. The larger volume of water thus held in the river would not be warmed so readily by the sun and the spray from the waterfall or cascade would cool the water somewhat by increasing evaporation. Another method would be *to make a deep channel*, so that the water would not be spread out so thinly as it is now. A third method would be *to raise the water level* during critical periods, from water stored by means of dams, as is necessary for creating artificial freshets.

SUMMARY

Salmon have died in both the Moser and the St. Mary rivers in summers of very low water. The principal deaths occurred at the time of day and on the days when the water temperature was highest, as well as in the part of the river with the highest temperature.

The salmon became insensitive to light and came to the surface, some of them congregating in cooler side waters with more or less complete recovery. When dying, there was loss of equilibrium and cessation of both swimming and breathing.

Those freshly in from the estuary died at about 29.5° and those long in the river only at about 30.5° C., showing the influence of acclimation.

Larger salmon died before grilse, but no parr died, although they were stimulated to migrate and to some degree lost their sensitivity to light, coming into shallow water. This indicates greater resistance in the young.

High temperature was clearly the principal cause of death, although in some cases removal of skin by sea lice and overexertion from being chased were at least contributory factors.

Experiments, with the temperature rising 1° C. every five minutes showed that the salmon parr died at from 32.9 to 33.8°, and in order according to size, the larger first, while young suckers (*Catostomus*) died at from 35.1 to 36.1°.

The fish freshly in from the estuary that died were brought in from the cooler tidal waters by rainfall after a long period of drought.

The place in the Moser river where the deaths occurred had the highest temperature, because the water was very shallow, which made it readily warmed by the sun, and without cascades, which would cool the water by increasing evaporation.

The time of day (afternoon) when the deaths occurred had the highest temperature as a result of heating by the sun's rays reaching a maximum at noon with a lag in the peak temperature of two hours or more.

The dates, when the deaths occurred, were determined by the highest temperature being reached at the ends of periods of several days with air in from the ocean and characterized by local absence of clouds and high moisture content in the lower layers, with a tendency for fog to creep in from the sea

in the late afternoon and evening. This gave only moderate daily amplitude in temperature, but with a general rise from day to day. For the air, there was rise in daily maximal temperature and dew point temperature.

Prevention of the mortality is seen as possible either through bringing the fish into the river early by means of artificial freshets, or by keeping down the water temperature in the lowland section through the construction of low dams to make deeper pools and cascades, through making a deep channel, or through raising the water level during critical periods with stored water.

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